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DIFFERENCES IN THE RECOVERY OF NITROGEN FROM AMMONIACAL AND NITRATE SOURCES IN THE RECLAIMED POLDERS OF THE ZUYDER ZEE

by J. J. LEHR

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Introduction. As early as 1849, plans were made to reclaim the Zuyder Zee, but it was not until 1918 that the Dutch Parliament approved the enterprise. In 1920 work was begun on draining the "Wieringermeer" polder, an area of 20,000 hectares in the north west corner of the Zuyder Zee and a little later, on building a 30 kilometer dam which was designed to convert the whole Zee into a freshwater lake. The "Wieringermeer" polder was brought under cultivation in the early thirties and, with the completion of the dam in 1932 (since when the Zuyder Zee has been officially called the Yssel lake) the other three polders envisaged in the plan could be drained. One of these, the "North East" polder, of 48,000 hectares was reclaimed in 1942. The greater part of the scheme however still remains to be tackled. This entails the draining of the "South East" and "South West" polders of 95,000 and 55,000 hectares respectively. Preparations for the draining of the "South East" polder are now being made. The whole draining enterprise will increase the area under cultivation in the country by about 10%. Fig. 1 illustrates the scheme.

From the time when the reclaimed soils of the "Wieringermeer" and later of the "North East" polder were brought under cultivation there has been evidence of considerable differences in the effect of ammoniacal as compared with nitrate nitrogen. Cases are common where the effect of the former is less than half that of the latter. S m e d i n g [8]) states that during the early cultivation period of the "Wieringermeer" polder, the average effect of nitrogen, applied as sulphate of ammonia did not exceed even one third of that of nitrate nitrogen. B o s m a [1]) mentions a provisional average value of three quarters for the comparative effect of ammoniacal nitrogen in the

"North East" polder but as he points out, there may be wide variations in this respect. The soils in question are almost invariably rich in calcium carbonate and according to present theory, these are just the soils which would respond well to ammoniacal nitrogen (e.g. sulphate of ammonia). Experience gained on older lands throws

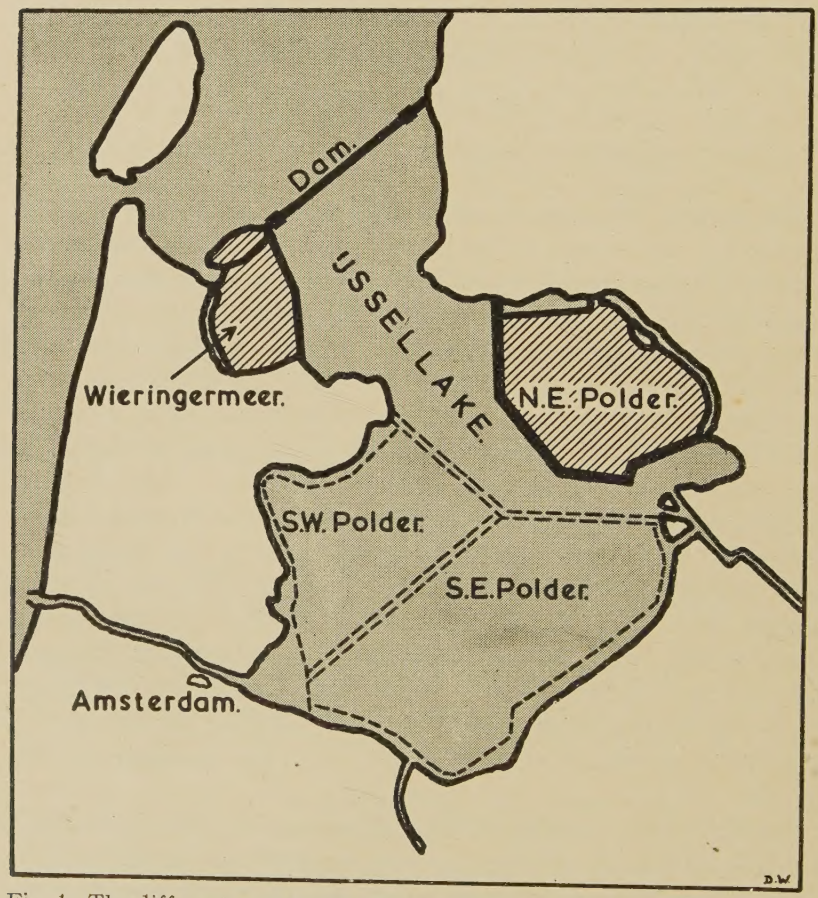

Fig. 1. The different recovery from ammoniacal and nitrate nitrogen in the reclaimed polders of the Zuyder Zee.

little light on the problem. In 1935, H a r m s e n [4]) suggested that ammoniacal nitrogen would be subject to volatization in the newly reclaimed soils but as far as we know, no evidence has been put forward to support this suggestion so that the explanation must be accepted as unconfirmed.

Experiment 1. During an excursion to the "North East" polder (June 19, 1947) under the guidance of Ir. W. A. B o s m a, we inspected one of his experiments (Section P 64) on the "Ramspol" sands where the above mentioned phenomenon was very marked. Samples of plants were collected for investigation with the object of finding some clue as to the cause of this very unequal nitrogen effect.

This long term field experiment was laid out to compare the effects of increasing applications of sulphate of ammonia and Chilean nitrate. In 1947 oats were grown. The rates of application were 0, 40, 80 and 100 kilos N/ha. At the time of sampling, the oats were still in the vegetative stage but were already showing marked differences in response to the two fertilizers. The plants treated with sulphate of ammonia were very much the poorer and generally showed a distinct pale yellow discoloration, closely resembling that symptomatic of N deficiency, even on the plots receiving the higher doses of N. The plants treated with Chilean nitrate on the other hand were of the normal green colour.

The dried plant material was analysed for Mg and N to see whether the discoloration of the plants treated with sulphate of ammonia could be attributed to an insufficient uptake of Mg (with defective chlorophyll formation) or to an inadequate N supply.

TABLE I

Content of nitrogen and magnesium in oat plants from field experiment at Ramspolzand (Section P 64)

kilos N per ha	Form of nitrogen	field A		field B	
		% N	m.e. Mg per 100 g dry matter	% N	m.e. Mg per 100 g dry matter
0		1.18	11.5	1.45	11.3
40	Ch.Nitr. . .	2.07	12.3	1.58	9.0
80	Ch.Nitr. . .	2.06	12.5	2.40	11.5
100	Ch.Nitr. . .	2.17	11.7	2.62	12.1
40	S. Amm. . .	1.22	10.7	1.29	9.4
80	S. Amm. . .	1.28	8.2	1.40	11.0
100	S. Amm. . .	1.22	9.4	1.31	9.1

Although the Mg content of the plants treated with sulphate of ammonia is on the whole lower than both that of the controls and those treated with Chilean nitrate (the average contents being 9.6, 11.4 and 11.5 m.e. Mg per 100 g dry matter respectively) the main

differences lie in the N contents. With one exception, the effect of increasing doses of ammoniacal N is negligible in comparison with that of nitrate N. Particularly striking is the fact that the N figures for the plants on the sulphate of ammonia plots do not differ materially from those on the plots receiving no nitrogen.

Taking the average values for the three N levels in each case, we have the following:

	A	B
Without dressing	1.18% N	1.45 % N
Sulphate of Ammonia	1.24% N	1.33 % N
Chilean nitrate	2.10% N	(2.20)% N

On the basis of determinations of dry weight and fresh weight of the plants and row length, the following rough estimate of total uptake by the crop of Mg and N per hectare can be made:

TABLE II

Yield of dry matter, magnesium and nitrogen in kg/ha of field experiment at Ramspolzand (Section P 64)

Kilos N per ha	Form of N	field A			field B		
		kg dry matter	kg Mg	kg N	kg dry matter	kg Mg	kg N
0		1174	1.6	13.9	478	0.7	6.9
40	Sulph.amm.	1543	2.0	18.8	1152	1.3	14.9
80	Sulph.amm.	1480	1.5	18.9	1435	1.9	20.1
100	Sulph.amm.	1521	1.7	18.6	1521	1.7	19.9
40	Ch.nitr.	3043	4.5	63.0	2217	2.4	35.0
80	Ch.nitr.	4261	6.5	87.8	2782	3.9	66.8
100	Ch.nitr.	3478	5.0	75.5	3565	5.3	87.4

Despite the approximate nature of this calculation it is obvious that the ammoniacal dressings lay far behind the nitrate dressings not only as far as the yield is concerned but also in respect of N recovery by the crop. This may be considered as the direct cause of poor plant growth (nitrogen hunger).

Experiment 2. In view of the fact that the field experiment at Ramspolzand represented a rather extreme case, it was felt desirable to investigate the phenomenon under under more general conditions.

In 1948, with the cooperation of Ir. B o s m a, a more comprehensive field experiment was planned, from which samples could be in-

vestigated to obtain more precise information as to the movements of the nitrogen. The experiment was set out by the "Directie van de Wieringermeer-Noord Oost Polderwerken" on a lime rich gritty clay field (Section N 17) according to the following lay out:

Each treatment was replicated three times as follows:

Plot No 1 — No dressing
No. 2: Chilean nitrate, 80 kg N/ha.
No. 3: Sulphate of Ammonia 80 kg N/ha.
No. 4: Chilean nitrate, 120 kg N/ha.
No. 5: Sulphate of Ammonia 120 kg N/ha.
No. 6: Chilean nitrate, 160 kg N/ha.
No. 7: Sulphate of Ammonia 160 kg N/ha.

No dressings of phosphate or potassium were necessary on this soil at the time.

Crop: Summer wheat, sown 17th March.

Dressings applied on 16th April (broadcast in the usual way, over the surface of the soil).

To study the trend of the N recovery by the crop, the plots were sampled at intervals viz.

18th May	early vegetative stage
1st June	late vegetative stage
16th June.	on earing
13th August.	fully ripened plants.

Along with the plants, soil samples were taken and transported to the laboratory the same day for the immediate determination of nitate and ammoniacal N content (see soil investigation).

Methods of analysis of soil and plant samples. The soil samples collected in the morning by the "Directie van de Wieringermeer-Noord Oost Polderwerken" were extracted in our laboratory on the same day so as to minimize the possibility of conversion in the samples.

Ammonia was determined colorimetrically with Nessler's reagent after extraction with a sodium acetate-acetic acid mixture (pH 4.8).

Nitrate was determined colorimetrically with phenoldisulphonic acid after extraction with water.

The plant samples were dried and their N content determined by the Kjeldahl semi-micro method, using selenium as the catalyst.

The author wishes to express his thanks to Miss A. J. M. Hoogstede who made the analyses.

Yields. Final Yields, as determined by the "Directie van de Wieringermeer-Noord Oost Polderwerken", are given in Table V. From these data the effect of ammoniacal nitrogen in relation to that of nitrate nitrogen can be approximately estimated to be slightly more than 50%, since 80 kg N in the form of nitrate was nearly sufficient for maximal grain yield.

160 kg N as Chilean nitrate was too high an application and caused a decrease in the final yield of grain.

TABLE III

Summary of the nitrogen content in summer wheat (as percentage dry matter) during the whole growth period

Date	no N dressing	Sulphate of Ammonia			Chilean Nitrate of Soda		
		80 kg N	120 kg N	160 kg N	80 kg N	120 kg N	160 kg N
18 May	2.70	3.07	3.35	3.68	4.39	4.78	4.69
	2.53	2.71	3.00	3.45	4.20	4.76	5.07
	2.45	3.24	3.26	3.46	4.02	4.88	4.88
Average	2.56	3.01	3.20	3.53	4.20	4.81	4.85
June, 1	1.92	1.99	2.05	2.27	2.42	2.52	3.66
	2.01	1.94	2.18	2.18	2.61	2.78	3.39
	1.63	2.12	2.13	2.32	2.37	3.17	3.64
Average	1.85	2.02	2.12	2.26	2.47	2.82	3.56
June, 16	1.19	1.22	1.42	1.39	1.31	1.95	1.89
	1.01	1.11	1.64	1.76	1.29	2.05	2.68
	1.51	1.68	1.64	1.87	2.05	2.32	2.51
Average	1.24	1.34	1.57	1.67	1.55	2.11	2.36
Aug., 13 *straw*:	0.36	0.32	0.30	0.37	0.38	0.39	0.62
	0.28	0.24	0.21	0.30	0.45	0.39	0.66
	0.21	0.25	0.28	0.33	0.29	0.47	0.54
Average	0.28	0.27	0.26	0.33	0.37	0.42	0.61
grain:	1.64	1.52	1.48	1.68	1.68	1.85	1.97
	1.49	1.47	1.50	1.63	1.61	1.84	2.15
	1.47	1.50	1.61	1.69	1.63	1.95	2.09
Average	1.53	1.50	1.53	1.67	1.64	1.88	2.07

Results of plant investigations; nitrogen recoveries. The N-content of the plants from the different plots, determined at the successive intervals, is given in tabel III. These data cover the whole plants, except those of the samples drawn on the 13th August. The average N-content of each object, calculated therefrom has been shown in fig. 2 which gives a clear

idea of the heavy fall of this content in the course of the growing period. The straw with its final low N-content lies in the same track. The major part of the nitrogen absorbed by the plant moves to the

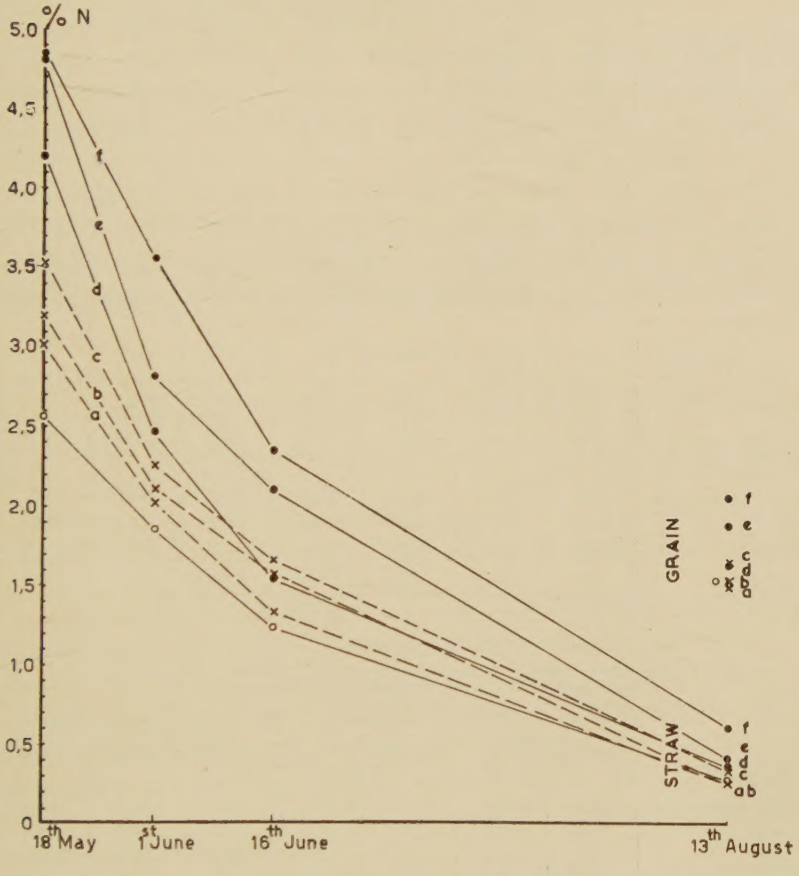

Fig. 2. Nitrogen content in summer wheat (as percentage dry matter) during the whole growth period.

o = no N dressing
a = 80 kg N/ha as Sulph. Amm.
b = 120 kg N/ha as Sulph. Amm.
c = 160 kg N/ha as Sulph. Amm.
d = 80 kg N/ha as Ch. Nitr.
e = 120 kg N/ha as Ch. Nitr.
f = 160 kg N/ha as Ch. Nitr.

grains, the N-content of which is about equal to the average content of the whole plant in its earing stage.

As was to be anticipated, the plants on the plots without nitrogen had the lowest N-content; ammoniacal nitrogen only produces a

TABLE IV

Calculated values of nitrogen recovery in kilos per hectare (on May, 18)							
	no N dressing	Sulphate of Ammonia			Chilean Nitrate of Soda		
		80 kg N	120 kg N	160 kg N	80 kgN	120 kg N	160 kg N
First replication	25.8	35.8	46.4	54.2	67.0	84.7	70.1
Second replication	25.7	31.4	39.2	52.3	51.3	80.2	91.1
Third replication	26.9	35.9	45.7	43.2	54.1	78.7	71.7
Average	26.1	34.4	43.8	50.0	57.5	81.2	77.6

TABLE V

Final yields in kilos per hectare (Aug., 13)							
	no N dressing	Sulphate of Ammonia			Chilean Nitrate of Soda		
		80 kg N	120 kg N	160 kg N	80 kg N	120 kg N	160 kg N
Straw:							
1st replication	4356	5445	7362	6435	6525	7407	7677
2nd ,,	4140	5742	5931	6462	7092	7587	7668
3rd ,,	4311	5751	5814	5976	7083	7605	7632
Average	4269	5646	6369	6291	6900	7533	7659
Grain:							
1st replication	2664	2871	3483	3555	3564	3546	3195
2nd ,,	2457	3087	3339	3942	3429	4014	3555
3rd ,,	2556	3312	3375	3312	3555	3330	3105
Average	2559	3090	3399	3603	3516	3630	3285

TABLE VI

Final nitrogen recovery in kilos per hectare (Aug., 13)							
	no N dressing	Sulphate of Ammonia			Chilean Nitrate of Soda		
		80 kg N	120 kg N	160 kg N	80 kg N	120 kg N	160 kg N
Straw:							
1st replication	15.7	17.4	22.1	23.8	24.8	28.9	47 6
2nd ,,	11.6	13.8	12.5	19.4	31.9	29.6	50.6
3rd ,,	9.1	14.4	16.3	19.7	20.5	35.7	41.2
Average	12.1	15 2	17 0	21.0	25.7	31.4	46.5
Grain:							
1st replication	43.7	43.6	51.5	59.7	59.9	65.6	62.9
2nd ,,	36.6	45.4	50.1	64.3	55.2	73.9	76.4
3rd ,,	37.6	49.7	54.3	56.0	57.9	64.9	64.9
Average	39.3	46.2	52.0	60.0	57.7	68.1	68.1
Straw + *Grain*:							
Average	51.4	61.4	69.0	81.0	83.4	99.5	114.6

slightly higher N-content depending on the amount of the N-doses. In the case of nitrate nitrogen the N-content reaches a much higher

level and it is worth while to point out that the single dose of 80 kilos N in the form of Chilean nitrate produced on an average even a higher N content than the double N dose (160 kg/ha) in the form of sulphate of ammonia, while the content of the grains turns out to be precisely equal for both objects.

It was originally the intention to make intermediate yield determinations to calculate the nitrogen uptake per hectare by the crop during the whole growth period. This has not been done, so that for a somewhat accurate estimate of the total nitrogen uptake we have only available the figures of the first sampling (for which samples covering plant rows of 3, 4 or 5 meters length had to be drawn; a yield estimate can be based hereon). Table IV and VI. These data can be summarized in the following averages of nitrogen recovery.

TABLE VII

Nitrogen Recovery in kg/ha on May 18 and August 13 (final stage)								
Kilos N	May 18				August 13			
	0 kg	80 kg	120 kg	160 kg	0 kg	80 kg	120 kg	160 kg
No N dressing	26.1	—	—	—	51.4			—
Sulph. Amm.	—	34.4	43.8	50.0	—	61.4	69.0	81.0
Ch. Nitrate	—	57.5	81.2	77.6	—	83.4	99.5	114.6

Accordingly, on this soil the recovery from ammoniacal nitrogen is substantially lower than that from nitrate nitrogen. Moreover, the figures make it clear that the nitrogen recovery is decided already in a very early stage. Taking into account the nitrogen uptake by the plots without N-dressing, we find that on August, 13 the extra nitrogen uptake on the dressed plots was not higher than that of May 18th, except in the case of the highest N dressings which show slightly higher figures.

Results of soil investigations. To determine the initial status of the soil, samples were collected on April 29th from the three control plots, receiving no N dressing.

The following values were obtained:

pH 8.2
Organic matter : 2.2%
$CaCO_3$: 9.5%
P soluble in water : 2
P soluble in citric acid : 11

Since even heavy dressings caused only slight increases in concentration in the soil, the investigation was confined to the fertilized plots receiving the highest N dosages. Both control and fertilized plots were first sampled on May 19th and subsequently on June 2nd, June 17th and August 23rd. The values for nitrate and ammonia contents are given in Table VIII.

Nitrate nitrogen was also tested for but was invariably found to be very low.

TABLE VIII

Soil analyses — averages of 3 replications — in p.p.m. N on soil *)						
Date of sampling	No N dressing		Sulph. of Amm.		Chilean Nitr.	
	NO_3–N	NH_4–N	NO_3–N	NH_4–N	NO_3–N	NH_4–N
April, 29	8	3	—	—	—	—
May, 19	— †)	— †)	6	9	25	6
June, 2	2	1	4	2	8	2
June, 17	1	2	0.4	1	5	1
August, 23	2	6	2	6	4	6

*) To give an idea of the variation in the values between individual plots, detailed determinations of May, 19 are shown below:

	NO_3–N	NH_4–N
Sulphate of ammonia . . .	8 — 6 — 4	10 — 8 — 8
Chilean nitrate	25 — 31 — 20	5 — 7 — 6

†) not determined

According to Table IV, the average values for N absorbed by the crop per hectare on May 18th was as follows:

	N absorption	Excess absorption on dressed plots
No N dressing	26.1 kg	
Sulphate of ammonia (160 kg N) .	50.0 kg	23.9 kg
Chilean nitrate (160 kg N) . . .	77.6 kg	51.5 kg

An attempt can now be made to draw up a balance sheet for nitrogen, beginning with an initial concentration for the fertilizer of 53 p.p.m. (i.e. 160 kilos N distributed over a cultivated furrow of 3,000,000 kg).

	Initial concentration	Excess absorbed by the crop p. ha	Calculated rest of dressing	Actually determined (nitrate & ammonia)
Sulph. of Amm.	53 p.p.m.N.	8 p.p.m.N.	45 p.p.m.N.	15 p.p.m.N.
Chilean nitr.	53 p.p.m.N.	17 p.p.m.N.	36 p.p.m.N.	31 p.p.m.N.

This balance sheet does not take into account the N present in the soil of the control plots on May 18th — this may be estimated at 3 to 5 p.p.m. The calculation also disregards any microbiological activity and assumes no leaching. Despite these omissions, the balance sheet figures for the concentration of active N in the case of the nitrate dressing, correspond fairly well whereas in the case of the ammoniacal dressing, there is a considerable deficit one month after fertilizing. Since the soil analyses suggest little nitrification in the plots treated with sulphate of ammonia, there arises the question of the disappearance of the ammoniacal N.

Discussion. The present investigation has shown that the inferior results obtained with ammoniacal nitrogen on the newly reclaimed Zuyder Zee polders is associated with low nitrogen recoveries, i.e. only a relatively small part of the ammoniacal dressing is taken up by the plants. There is some evidence that the N recoveries are comparatively lower than the corresponding yields; this suggests that low yields are accompanied by low protein contents.

Calculations from the results of Experiment 2, indicate an average N recovery value of 40% for the nitrate dressings irrespective of the dosage and values of 12, 15 and 19% for applications of 80, 120 and 160 kg N/ha respectively in the ammoniacal form.

The uptake values at an early stage (June 19) determined in Experiment 1 (Ramspolzand) show for Chilean nitrate a recovery of 74% and for sulphate of ammonia a recovery of 10% (calculated on the basis of 80 kg N/ha dressing). It may be argued that these latter values are but very rough approximations and that they might improve during the course of growth but there is no evidence for a delay in the effects of ammoniacal nitrogen and a subsequent recovery. Soil analyses in fact indicate that ammoniacal N disappears altogether.

These findings appear to be incompatible with the general belief that ammoniacal N is an excellent fertilizer for lime rich clay soils and for overlimed sandy soils. It has been occasionally suggested, as a tentative explanation, that microbiological activity in these young polder soils has not yet reached maturity and that consequently nitrification takes place only very slowly. Such an explanation however would only be applicable if nitrates were the only form of N assimilated by plants and it is well known from physiological

experiments that cereals can also assimilate ammoniacal nitrogen provided that a certain amount of nitrate is also present. The above experiments show in fact that little nitrification takes place, but even so it is of little importance since a substantial fraction of the added ammoniacal N disappears from the soil before the growing plant can absorb it.

As mentioned before, H a r m s e n [4]) dealing with the "Wieringermeer" polder, attributed this phenomenon to volatilization of part of the ammonia from ammonia containing fertilizers such as sulphate of ammonia and nitrate of ammonia (kalkammonsalpeter) under the influence of high contents of calcium carbonate and general base richness of these soils in conjunction with low carbonic acid production. As far as we know this explanation was never confirmed by him by factual data. It is, however, relatively easy to demonstrate that ammonia is released when such a soil is mixed with sulphate of ammonia under moist conditions *).

In some provisional laboratory determinations made by Mr. J. C h. v a n W e s e m a e l, air at 20°C was passed at a moderate rate over a 2 cm layer of soil (sampled from experiment 2) to which 100 mg $(NH_4)_2SO_4$ (= 22.5 mg N) and 4 g water had been added per 50 g soil (This corresponds to a dressing of 140 kilos N/ha distributed to a depth of 2 cm). After an initial reaction period of a few hours ammonia in quantities varying from 0.11 to 0.18 mg N per hour, was released. Calculated per day, this amounts to at least 2.5 mg N, suggesting therefore that at least 15 kilos N/ha would be lost daily under comparable conditions.

These experiments give some idea as to the nature of the ammonia losses although laboratory results are not quite applicable to field conditions since in field practice so many factors such as temperature, soil moisture, wind velocity, sunshine etc., are beyond control.

Volatilization of ammonia in this way has been known for some time, attention having been brought to this phenomenon even in the early days of agricultural chemistry. D i b b i t s [2]) made a chemical investigation of the phenomenon as far back as 1874, while W a g n e r amongst others, demonstrated volatilization in 1903 using

*) Since the time when this paper was presented for publication Van Schreven, at the IVth Int. Congr. of Soil Science in Amsterdam, communicated a paper on laboratory investigations, which gave straight evidence of ammonia volatilization on the reclaimed Zuyderzee soils (Transactions, I (1950) 259—261).

dishes of soil dressed with sulphate of ammonia. Schneidewind [7]) carried out a number of pot experiments with different soil types and showed on the basis of crop analyses that the N recovery from nitrate and ammoniacal N differed little on lime deficient soils but that on lime rich soils, ammonia had a distinctly inferior effect, particularly when the sulphate of ammonia was not worked into the soil but applied superficially. Geerts [3]) also states that a superficial application of sulphate of ammonia to lime rich soils in the cultivation of cane sugar may result in N losses but not when the normal practice of working the fertilizer into the soil is adopted. More recently Jewitt [5]) has investigated the loss of ammonia from alkaline soils under laboratory conditions. Kappen *et al.* [6]), Steenbjerg [9]) and Tovborg-Jensen and Betsy Kjær [10]) have also studied this phenomenon under laboratory conditions and have investigated the factors influencing the volatilization of ammonia from soils containing calcium carbonate.

It is rather remarkable that in Holland, at least in agricultural circles, this aspect of nitrogen fertilization has received no attention in the past but that it only came to be recognized as a problem following practical experience in the Zuyder Zee polders. Ammonia volatilization must be considered to be one of the principal causes of the low N recoveries in the "Wieringermeer" and "North East" polders. It may also explain the poor results obtained with farmyard manure on these soils.

The occurrence of this phenomenon raises a number of questions: what are the conditions which enhance or reduce ammonia volatilization, how can the losses be controlled and why has this phenomenon not been established on the older lime rich soils cultivated in Holland? A possible explanation of the last point may be that in the older soils nitrification proceeds at a much greater rate than in the newly reclaimed soils so that the danger of ammonia losses is substantially reduced.

A further study of this phenomenon is most desirable since it is of more general importance in furthering our knowledge of the N economy of the soil.

The author wishes to express his gratitude to the "Directie van de Wieringermeer-Noord Oost Polderwerken" and particularly to Ir. W. A. Bosma for their kind co-operation in this work.

Summary

An investigation has been made into the unequal effects of nitrate and ammoniacal nitrogen in the reclaimed "Zuyder Zee" polders. Plant analyses have shown that the crop recovers much less nitrogen from ammonia dressings than from nitrate dressings.

Soil investigations showed clearly that even only a few weeks after fertilisation, a considerable proportion of the ammonia nitrogen had disappeared from the soil without any appreciable nitrification having taken place.

Ammonia volatilization under the influence of the lime richness of the soil is assumed to be the explanation for this loss- the phenomenon being readily demonstrated by simple laboratory tests.

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LITERATURE

1) Bosma, W. A., De resultaten van de onderzoekingen omtrent de stikstofbemesting in de Noord Oost Polder. Directie van de Wieringermeer (N.O.P. werken), No. 5, Ontwikkelingsdag te Markenesse op 4 December 1946.

2) Dibbits, H. C., Über die Dissoziation von Ammoniumsalze in wässeriger Lösung. Z. anal. Chem. **13**, 395–408 (1874).

3) Geerts, J. M., Samenvattende bewerking van de resultaten van proefvelden bij de rietcultuur op Java. Vergelijking van Chilisalpeter tegenover Zwavelzure Ammoniak (t.e.m. 1914). Arch. Suikerind. **24**, 929–1001 (1916) I.

4) Harmsen, G. W., De microbiologie der Zuiderzee-gronden. Landbouwkund. Tijdschr. **47**, 852-875 (1935).

5) Jewitt, T. N., Loss of ammonia from ammoniumsulfate applied to alkaline soils. Soil Sci. **54**, 401–409 (1942).

6) Kappen, H., Scheng Tscheng-Jen, Nickolay W., Wienhues, W., Zur Verflüchtigung von Ammoniak aus Lösungen von Ammonsalzen. Bodenkunde u. Pflanzenernäh. **31**, 223–244 (1943).

7) Schneidewind, W., Die Ernährung der landwirtschaftlichen Kulturpflanzen, Berlin (1928), p. 81.

8) Smeding, S., Het in cultuur brengen van Zuiderzeegronden, voorheen en thans. Landbouwkund. Tijdschr. **47**, 797–826 (1935).

9) Steenbjerg, F., Ammoniaktab fra kvaelstofholdige Handelsgødninger ulbragt paa Agerjord; Tids. Planteavl, **48**, 516–543 (1942/43).

10) Tovborg Jensen S. and Betsy Kjær, Kvælstoftab ved Ammoniakfordampning efter Gødskning med Svolvsur Ammoniak; Tids. Planteavl **51**, 666–711 (1948).

11) Wagner, P., Die Düngung mit schwefelsaurem Ammoniak und organischen Stickstoffdüngern im Vergleich zum Chilesalpeter. Arb. Deut. landw. Ges. **80**, 14 (1903).

12) Vide, *for example*: Jaarverslag van de Vereniging voor Bedrijfsvoorlichting in de Wieringermeer van 1943, pag. 4 and 35, and: Verslag over de landbouwproefvelden van Prof. v. Bemmelenhoeve in 1942 and 1943, p. 81.

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STUDIES WITH SWEET CORN III

THE PRIMARY EFFECTS OF TREATING SEEDS WITH ULTRASONICS

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Introduction. Several reports have indicated that it is possible to stimulate growth in plants by treatment with ultrasonics. Istomina and Ostrovsky[7) 8)] claim that treating seeds of certain crop plants, *e.g.* peas and potato tubers, with ultrasonics at a frequency of 400 Kc sec before planting causes early maturity and increased yields. Newcomer and Wallace[10)] state that resumption of growth in root-tips of *Narcissus* following ultrasonic treatment at 400 Kc sec was often accompanied by marked stimulation which after several days produced longer roots than the controls. They also reported stimulation and early maturation of other (un-named) plants after ultrasonic treatment of apical meristems. Recent statements in the English and American lay press (Anon.)[1)], report that yields of hybrid corn (maize) can be increased fifteen per cent. by treating the seeds with ultrasonics. However a detailed statistical study of any such stimulation effect seems to be lacking; the necessity of proving that an apparent stimulation effect is in fact statistically significant cannot be overemphasised.

Wallace, Bushnell and Newcomer[15)] have noted cytogenetic variations in *Drosophila* and *Helianthus* induced by ultrasonics. This report is not in accord with the work of Hersh, Kerrer and Loomis[6)] who obtained no conclusive evidence

that the mutation rate in *Drosophila melanogaster* is increased by ultrasonic treatment. Conger [3]) using *Tradescantia* buds found that sonic energy at 9.1 Kc/sec applied simultaneously with X-rays increased the chromosomal aberrations produced in the ratio 1.3 : 1.2. He also showed that ultrasonic treatment alone did not produce aberrations. More recently Newcomer and Wallace [10]) exposed *Narcissus* root tips to various durations of ultrasonic waves at a frequency of 400 Kc/sec and compared the chromosomal and nuclear aberrations with those produced by X-rays, mustard gas, and nucleic acid starvation.

It was decided therefore to re-investigate some of the effects which various dosages of ultrasonic waves might have upon quantitative characters of maize to determine their practical application to agriculture. The experiment was so designed to allow detailed statistical study of possible stimulation effects on the materials used.

In general the treatment of biological material in seed form permits application of more severe experimental treatments without producing lethal effects than would be possible with the living material in the turgid state. The major effects of high intensity ultrasonics applied in a liquid medium are due to cavitation (E. W. Harvey) [5]), and are not critically dependent upon the frequency used, but vary with the intensity of the ultrasonic beam.

The plant material. The genetical constitutions of the two sweet corn lines studied are as follows. Inbreds were used as they would be expected to be reasonably homozygous. They were *C 4*, early maturing, and *P 39*, somewhat later and with flat regularly shaped seeds: *P 39* is widely used as mother parent for *Golden Cross Bantam* and both have a reputation for being uniform in America.

Inbreds were used in preference to vigorous F1 hybrids as on selfing treated plants there would be no confusion between the effects of ordinary F2 gene segregations and effects due to ultrasonics. Hybrids between treated and untreated plants could also be made with a tester inbred to see if there was stimulation of hybrid vigour. Any genetical or environmental effects resulting from ultrasonic treatment would be readily recognised. Inbreeding these reasonably pure lines would release any mutants induced.

C 4 was used only in laboratory tests and *P 39* was used both in preliminary investigations and in field studies. As *P 39* had not

previously been grown in England it was not known at first whether it would mature sufficiently early for the seeds to ripen before autumn frosts.

Apparatus. The ultrasonic apparatus was specially developed at King's College for the treatment of biological material under controlled conditions of temperature and high uniform ultrasonic intensity.

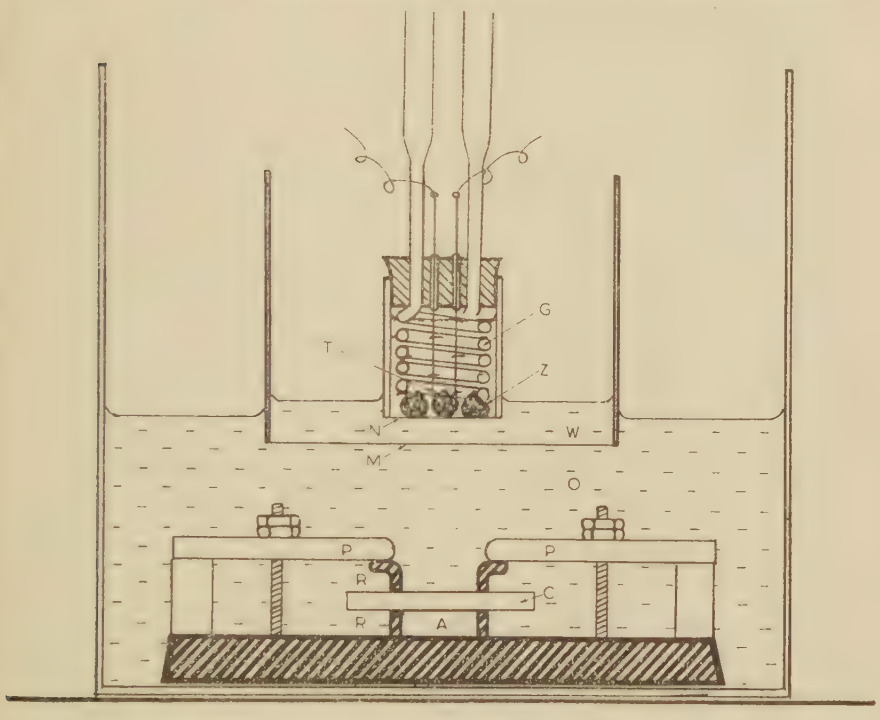

Fig. 1. The ultrasonic generator and treatment vessel.

The generator was of the piezo-electric type, in the form of a quartz crystal (X-cut) driven at its fundamental frequency of 1 Mc sec. The output of a parallel-fed Hartley oscillator, using two type CRC 813 valves in parallel, was matched to the crystal through a high-frequency output transformer. The crystal was 1 in. in diameter with disc electrodes of aluminium 1.5 sq. cm in area, which had been deposited centrally on opposite faces by evaporation *in vacuo*. It was mounted as in Fig. 1 (letter C), clamped by means of perspex

strips P, P between two brass contact rings R, R and backed by a reflecting air space A. A parallel beam of ultrasonics was transmitted through the transformer oil medium O, across a cellophane membrane M into water W, and across a second cellophane membrane N forming the bottom of the treatment vessel containing the seeds Z in water. Little power ($\sim 2\%$) was lost at the cellophane boundaries.

Full details of the oscillator circuit, ultrasonic techniques, and the calorimetric method employed to measure the ultrasonic intensities to which the seeds were exposed, are given by Selman and Wilkins[11]).

Ice-cooled water was pumped through the cooling spiral G of the treatment vessel. A thermojunction T was embedded at the centre of a control seed so that its temperature could be continuously followed during the experiments. It was assumed that the temperatures thus recorded were similar to those at the centres of seeds treated alongside.

Four or five seeds were treated at a time in distilled water, and were placed with their embryo sides towards the ultrasonic generator. During treatment, if the seeds were left for for thirty minutes in distilled water their uptake of water was approximately ten per cent of their own weight. This figure was not considered serious, and was found to be not sensibly reduced if the water was replaced by a sugar solution with an osmotic pressure of 20 atmospheres.

Preliminary experiments. A series of preliminary experiments were conducted in which all seeds after treatment were wrapped in a damp rag-doll seed tester (Burt, Biggar and Trout)[2]) and placed in a temperature control box maintained at 20°C. to determine germination which usually occurred after about four days.

These experiments showed that quite heavy treatments did not stop germination; seeds could be left in distilled water maintained at 50°C for an hour and still germinate. Germination was prevented if the temperature of the waterbath was allowed to rise to 60°C. Provided the temperature at the centres of seeds did not rise above 50°C, ultrasonics of the highest intensities could be applied without preventing germination, although after most severe treatments it was sometimes delayed by a day or so. To keep the material below the temperature limit, the highest ultrasonic intensities

could only be applied in a series of short bursts between intervals for cooling, even at the maximum rate of cooling which could be applied to the water medium.

The final course of treatments given in the main experiments is shown in Table I, and was designed to allow comparison between:

(1) The effects of ultrasonic treatment at a high intensity (35 watts/cm^2) and at lower intensity (10/watts/cm^2);

(2) The effects of ultrasonic treatment at the high intensity for a longer and shorter period;

(3) Genuine ultrasonic and purely thermal effects;

(4) All the above and the untreated controls.

The heat treatments were purposely more severe than the corresponding heating effects in the ultrasonic experiments to make the distinction (3) more obvious.

After treatment each seed was carefully dried on filter-paper and subsequently planted at the John Innes Horticultural Institution, Merton.

From the temperature rise of the seed during treatment and its cooling curve it was estimated that the seeds absorbed about 20% of the incident radiation to which they were exposed.

Field experiments. Table I also shows how treated *P 39* seeds and their controls were divided into two groups for planting out in two separate latin squares. Twenty four seeds were used for each treatment. In the first group seeds from each of the three treatments and the control were divided into four batches of six seeds each. In the second group there were three batches of eight seeds each. Each batch was sown on the 22nd. May, 1948 as a row in the appropriate position of its latin square, thus:

Square 1.	Square 2.
D B A C	
C A B D	F E G
B C D A	E G F
A D C B	G F E

(The letters refer to treatments and controls given in Table 1).

Rows were spaced two feet apart and seeds planted singly at one foot intervals along a row. Guard rows of *Golden Cross Bantam* surrounded both latin squares and inbred *C 13* acted as guard row between the two squares.

TABLE I

Ultrasonic treatment of maize

24 seeds of inbred *P 39* used for each treatment

Square I (*treated 13.5.48*)

Symbol	No: of seeds used	Ultrasonic Time	Treatment Time	Temp.	Intensity of U.S.	Notes
A	24	5 mins.	30 mins.	32°C (Peak)	35 Watts/cm²	(10 secs. burst of ultrasonics) × 30 (+ 50 secs. cooling)
B	24	30 mins.	,,	32°C (Steady)	10 Watts/cm²	Ultrasonics ran continuously
C	24	—	,,	50°C (Steady)	—	No ultrasonics – (Temp. control in water at 50°C
D	24	—	,,	18°C (Steady)	—	No ultrasonics – Control in water at 18°C

Square II (*treated 14.5.48*)

Symbol	No: of seeds used	Ultrasonic Time	Treatment Time	Temp.	Intensity of U.S.	Notes
E	24	15 mins.	60 mins.	40°C (Peak)	35 Watts/cm²	(15 secs. burst of ultrasonics) × 60 (+ 45 secs. cooling)
F	24	—	,,	50°C (Steady)	—	No ultrasonics (Temp. control in water at 50°C
G	24	—	,,	18°C (Steady)	—	No ultrasonics (Temp. control in water at 18°C

Field germination Table II gives the actual germination measured by emergence above soil level, for all treatments and controls. Controls D and G germinated 100% and 87% respectively; this is high for English conditions. The heat treatments C and F gave 83% and 71%; these are still reasonably good. The low intensity ultrasonic treatment B gave 79% germination, while the high intensity ultrasonic treatments A and E gave 50% and 54% respectively.

The high intensity ultrasonic treatment seems to have produced decreased germination under field conditions while the low intensity ultrasonic treatment produced no appreciable effect on germination. Analyses of variance were made on the data for maximum germination from both latin squares. The mean square for treatments compared with that for error gave a significant probability of p at the 1% level in square 1 and at the 5% level in square 2.

TABLE II

Field germination of *P 39* (Sown 22/5/48)

Treatment:		Experiment 1								Experiment 2					
		A		B		C		D		E		F		G	
Days from sowing	Date	Germination Actual	Rate %	Germination Actual	Rate %	Germination Actual	Rate %	Germination Actual	Rate %	Germination Actual	Rate %	Germination Actual	Rate %	Germination Actual	Rate %
13	4/6	0		0		0		0		0		0		0	
14	5/6	0		0		0		0		0		0		0	
15	6/6	0		0		0		0		0		0		0	
16	7/6	9	75%	14	73.7	15	75	18	75	11	84.6	15	88.2	16	76.2
17	8/6	11	91.7	18	94.7	19	95	23	95.8	12	92.3	17	100	18	85.7
18	9/6	12	100	18	94.7	20	100	23	95.8	12	92.3	17	100	18	85.7
19	10/6	12	100	19	100	20	100	24	100	12	92.3	17	100	19	90.5
20	11/6	12	100	19	100	20	100	24	100	12	92.3	17	100	20	95.2
21	12/6	12	100	19	100	20	100	24	100	13	100	17	100	21	100
Germination		50.0%	—	79.2%	—	83 3%	—	100%	—	54.2%	—	70.8%	—	87.5%	
Proportion		**.50**		**.79**		**.83**		**1**		**.62**		**.81**		**1**	

(Rates refer to percentages of the total germination for each treatment).

To determine the rates of germination the number of seeds which had germinated at each of a series of dates was expressed as a percentage of the total number which germinated after each treatment, and the results were plotted against time. Generally, germination rates were similar throughout.

There were three albino seedlings which died early. One occurred in control A and two in the heat treated F. Albinos occur occasionally in inbred lines; here they appeared in seedlings which did not have ultrasonic treatment and were due to spontaneous mutations or other causes. No mutations or abnormalities occurred in any plant grown in the two squares.

In England maize at the emergence stage suffers severely from damage by frit-fly and many of the seedlings showed signs of attack. Ravages of the flies possibly upset growth at the early stages, especially that of the controls which appeared to be more severely damaged, but later the plants recovered.

Growth. The heights of each plant were measured at frequent intervals during growth. As seed germination of some treatments had been poor, there were often few plants in a row. The data were therefore first analysed on the means of all plants from each treat-

ment. Fig. 2 is based on the mean heights. Plants in square 1 which recived ultrasonic treatment were somewhat taller in comparison with controls. The mean rations of heights for the heat treatment and the low intensity treatment B were similar. Differences of growth were not so different in square 2 and here controls and heat treatment were similar.

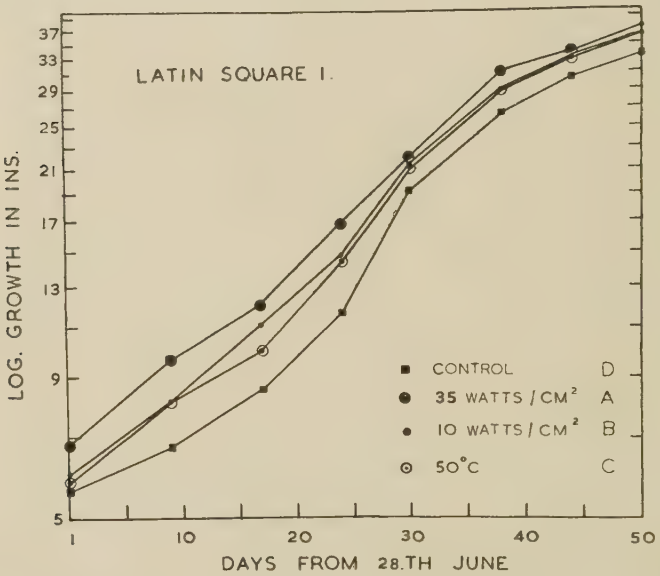

Fig. 2. The growth rate of *P 39* inbred sweet corn in latin square one. The seeds had previously received a high intensity (A) or low intensity (B) ultrasonic treatment, or heat treatment (C) or were left untreated (D). The data for each date were obtained from the *means* of all plants in each group. Statistical analysis showed that there were no significant differences between each group.

Even though *P 39* is an inbred line there was possibly some variation in seedling vigour and ultrasonic treatment may have killed off weaker seedlings. Their counterparts would still be growing in the controls and so would lower the mean heights of the control population throughout the grand period of growth. Adjustment for this possible source of error by weighting can be made after deletion of the values for weaker plants in those with higher germination. The means would then be based on equal numbers of vigorous plants for each treatment. This correction was made for the data

of 16th and 23rd July. The values of the means rose as contributions of weaker plants were eliminated (Table III). By calculating the ratios of the means of treated and untreated material for a particular number of plants, comparisons can be made. In the 16th July data the means for all treatments in square 1 are slightly greater than the controls but in square 2 they are almost the same. For the 23rd July data in square 1 the means are slightly in excess for treatments but not so much as for the previous week. In square 2 the controls were somewhat taller than treated plants.

TABLE III

			Adjusted Mean Height in ins.									
			16th July Means					23rd July Means				
		No. Germinated	24 plts	Top 19 plts	Top 15 plts	Top 11 plts	Top 6 plts	24 plts	Top 19 plts	Top 15 plts	Top 11 plts	Top 6 plts
	A	11	—	—	—	11.9 (1.01)	15.1 (1.17)	—	—	—	16.8 (0.99)	21.6 (1.16)
uare 1	B	16	—	—	11.6 (1.08)	12.0 (1.02)	14.7 (1.14)	—	—	15.6 (0.99)	17.7 (1.04)	20.3 (1.09)
	C	19	—	9.9 (1.16)	11.3 (1.06)	12.3 (1.04)	13.8 (1.07)	—	14.4 (1.02)	16.6 (1.06)	18.3 (1.08)	20.7 (1.11)
	D Control I	24	8.5	9.8 (1)	10.7 (1)	11.8 (1)	12.9 (1)	12.2	14.1 (1)	15.7 (1)	17.0 (1)	18.7 (1)
uare	E	12	—	—	—	10.6 (0.89)	13.1 (0.98)	—	—	—	16.0 (0.91)	18.7 (0.96)
	F	14	—	—	—	11.1 (0.93)	13.3 (1.00)		—	—	15.3 (0.87)	18.0 (0.93)
	G Control II	20	—	9.5	10.8	11.9 (1)	13.3 (1)	—	13.8	15.9	17.6 (1)	19.4 (1)

(Numbers in brackets refer to the ratio of means to controls)

To determine more critically whether plants from treated seeds have increased vigour, analyses of variance based on the latin square data were made for both squares at both dates. In each of the four analyses the mean square for treatments compared with the error mean square gave a variance ratio with a probability of p at the

20% level. Thus there were no genuine effects influencing growth due to either ultrasonic or heat treatment.

Flowering. Records were made of the dates when the first plants flowered in each row of both latin squares. Both tasselling and silking dates were taken. Owing to frit-fly damage, tasselling dates were not so reliable as silking dates, although under normal conditions these two dates and ear maturity are highly correlated (Singleton) [12].

TABLE IV

Flowering times
(Measured by Mean Date first flowers appeared)

	Square 1				*Square 2*		
	Treatment			Control	Treatment		Control
	A	B	C	D	E	F	G
Tasselling time . . . . . (Days after 23.8.48)	5.5	5.0	5.1	5.1	5.0	6.3	6.0
Silking time . . . . . (Days after 26.8.48)	5.5	5.1	5.0	7.0	9.0	7.3	5.0

Table IV gives the means of the first flowering dates in both squares. Analyses of variance were made on both sets of data. In square 1 tasselling time data gave a variance ratio of error mean square to treatment mean square of 23.27 with probability $p = 0.01 - 0.05$. On the other hand, silking time gave a variance ratio of error mean square to treatments mean square of 2.92 with probability $p = 0.20$. As silking dates were more trustworthy than tasselling dates the second analysis is more decisive.

In square 2 the error mean squares compared with with the treatments mean squares gave values of 14.84 and 1.50 for tasselling and silking dates respectively. Here $p = 0.20$ for both data. Ultrasonic treatment therefore did not influence the maturity of sweet corn.

Tillers. The tiller number of a maize inbred line is reasonably uniform in England. Although date of sowing influences the manifestation of tiller number, this is characteristic for any one sowing. The number of tillers of each plant in both latin squares were counted. The means for the total number of plants for each treatment are given in Table V.

The controls in both experiments were somewhat lower than for

TABLE V

Tillering						
Square 1 Treatment			Control	Square 2 Treatment		Control
A	B	C	D	E	F	G
4.60	4.13	4.17	3.70	4.17	3.93	3.78

(Figures refer to mean tiller number based on all plants)

ultrasonic and heat treatments. Analyses of variance were made on the two sets of latin square data. In both, the mean squares for treatments compared with the error mean square item gave probability values of p above the 20% level. Thus neither ultrasonic nor heat treatments had had a genuine effect on tiller number.

Leaves on main stem. Like a tiller number, the number of leaves on the main stem is a useful index of the behaviour of a quantitative character in maize. When the plants had flowered and were fully grown, the number of leaves on the main stems of each plant was counted. The means over all plants in a treatment are given in Table VI.

TABLE VI

Mean number of leaves on main stems						
Square 1 Treatment			Control	Square 2 Treatment		Control
A	B	C	D	E	F	G
9.4	9.1	9.3	9.2	9.2	9.9	9.2

Analyses of variance of data of both latin squares indicated that treatments had had no significant affects on leaf number as the probability p was greater than the 20% level in both squares for treatment mean squares compared with error mean squares.

Breeding results. All seedlings which germinated grew into normal healthy plants. There were no mutants other than the three albinos already noted. Ears were bagged before silking and numerous self pollinations and crosses made with *C 13* and *P 51* inbreds. Unfortunately *P 39* proved too late for English climatic conditions. Although four ears were selfed in each treatment and control, they failed to mature seed properly. Only a few ears were harvested by the end of October. These ears were still immature and, although dried in a warm greenhouse, produced mostly mouldy and shrivelled seeds. Control ears were just as poor as treated ones. A few ears were

salvaged and their seeds sown. These all failed to germinate except for four from A and one seedling each from E and F. There were several from the cross of *C 13* with C, a heat treatment which were all normal.

One A seedling was minute, another had leaves with one, two or three midribs and one had leaves paired instead of alternate. The other A seedling was normal. The E seedling was normal while that from F was of weak habit. The cytology of these seedlings will be discussed elsewhere.

Failure to germinate could not be attributed to ultrasonic treatment as all ears were well fertilised when picked. Although properly set, they were not sufficiently ripe for correct harvesting. Poor germination was thus due to the poor state of the seed at sowing due to difficulties of properly harvesting and drying *P 39* in England.

A re-investigation could be made with a pure line when one is known to ripen its seeds out-of-doors satisfactorily.

Discussion. These experiments show that reduced germination is the only genuine affect after field sowings following ultrasonic treatment of sweet corn seed. This loss of germination is not due to lethal effects produced by ultrasonic treatment, since in the rag-doll tests equivalent doses only slowed down the rate of emergence of plumules and radicles. The effect must be due to minute breaks caused by cavitation rupturing the dry, taut, testas covering the embryonic areas upon which the waves were directed. In cold soil these breaks allow easy access of pathogenic fungi, such as *Pythium*, which enter and kill off seedlings before they emerge above soil level (cf. T a t u m and Z u b e r) [14]. Following treatment, the delayed emergence in cold soil also encourages seed rotting by the same pathogens.

In America, corn-belt farmers pay great attention to field germination; the poor germination of F1 hybrid seed from inbreds delayed their use as hybrid corn. Any artifact which reduces germination by even a small amount, (and the above experiments show that ultrasonic treatment at 35 watts/cm^2 reduces field germination by 50%), would not be acceptable. Hence this factor alone would hinder the agricultural application of ultrasonics to maize improvement. Furthermore, as germinaton falls off, it would be difficult to expect higher yields per acre from a given amount of seed and the loss

would have to be compensated by heavier sowing rates. This means that farmers would have not only to pay more for seed treated with ultrasonics but also to buy a greater quantity than previously.

Measurements on growth and flowering, measured by first silking dates, indicated there were no significant differences between the various treatments and controls. These findings are in contrast to those obtained by Istomina and Ostrovsky[7)8)] and Newcomer and Wallace[10)] using other species. That treatment at 35 watt cm^2 resulted in 50% decreased germination shows that any experiment under field conditions employing even larger doses would be unlikely to serve a useful purpose. Indeed treatment at lower intensities might therefore be deemed more likely to produce stimulation effects. It is all the more significant that treatment at 10 watts cm^2 while not markedly reducing germination still produced no significant stimulation.

Although it was not possible to compare yields, there was no visual indication of increases in the treated rows. The increases previously claimed could be obtained by (1) increased ear weight (2) more ears per plant (3) bigger ears with heavier seeds (4) more seeds per ears (5) more ears ripe at the critical harvest date. Now while hybrid sweet corn produces several ears per plant, hybrid field corn has been bred to yield one ripe ear per plant *and no more*; therefore it may be inferred that the claimed increased yields were due to an increase in size of the individual ears. Yet two sensitive quantitative characters such as leaf and tiller number were unaffected by ultrasonic treatment. Hence one might expect that the quantitative characters controlling the growth of ears, *i.e.* that part of the same group of polygenes controlling growth, would also be unaffected. Therefore it is difficult to see how the increases occurred.

No plant grown from treated seeds in the above experiments was phenotypically abnormal. Any cytological effects produced by ultrasonics, such as have been reported by Newcomer and Wallace[10)], might be expected to impair rather than enhance yield. If any effects were produced they might be expected to resemble those of X-rays which Stadler[13)] considered valueless in plant breeding.

Now in Iowa the average increase in maize yields by conversion to double cross hybrids from open pollinated varieties is about 50%. Any further increase by the use of inbreds with improved combining

ability will become progressively slower. Hence an improvement of 15% in yield by ultrasonic treatment would be welcome. It has been observed by Gustafsson [4]) for barley, an inbreeding species, that dihybrids of two chlorophyll mutants (AaBb) had an increased kernel weight of 15% compared with normal (AABB) types. Hence it is feasible that if ultrasonic treatment of maize inbreds produced mutations, i.e. short deficiencies, in hybrid combination they might produce increased vigour over and above that due to restoration of heterozygosity. Jones [9]) has also found in maize that recessive mutations controlling degenerative changes show heterosis when crossed to normal lines from which they originate. Thus the bearing of this phenomenon on the application of ultrasonics to heterotic effects must not be ruled out.

Although the evidence points against a direct effect of increasing yields by ultrasonic seed treatment, there is thus still a possibility of obtaining heterosis following hybridisation of lines carrying induced mutations. This application of ultrasonics would only be feasible when improvement of hybrid corn by selection of better combining inbred combinations has reached its limit. Except for the United States no other area has approached this position so that at present ultrasonics seem to have little value for plant improvement. At present, X-rays are more reliable for inducing mutations.

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Summary

1. An investigation was made on the effects of treating inbred sweet corn seed with ultrasonics following reports of stimulation of other plants and of 15% increased yields from hybrid maize after ultrasonic wave treatment.

2. Preliminary laboratory experiments were made with inbreds *C 4* and *P 39*. Ultrasonics of the highest intensities at a frequency of 1 Mc/sec. did not prevent germination in a rag-doll seed tester provided the internal temperature of seeds did not rise above 50°C during treatment.

3. For field studies, seeds of *P 39* were given doses of 35 watts/cm^2 for 5 and 15 minutes, 10 watts/cm^2 for 30 minutes, and heat treatments at

50°C for 15 and 30 minutes. Controls were kept at room temperature (18°C). Sowings were afterwards made in two latin square blocks.

4. Controls gave the best germination in the field. For heat-treated seeds and for the 10 watt/cm^2 treatment it was 0.8 : 1, at 35 watts/cm^2 it was 0.5 : 1 and 0.6 : 1 for 5 and 15 minutes duration respectively.

5. All plants were phenotypically normal except for three albinos in the controls.

6. Statistical analyses were made on earliness, heights, tiller numbers and number of leaves on main stems. There were no statistically significant differences between treated and untreated material, except for a significant difference in first tasselling dates probably due to damage by frit-fly.

7. Ultrasonics have little value in commercial maize production owing to lowered germination. They might produce additional heterosis by producing mutations controlling degenerative changes. Widely publicised statements on the value of ultrasonics in agriculture are doubted.

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LIST OF REFERENCES

1) Anonymous, Ultrasonics. Life Magazine, 5th Jan. (1948).
2) Burt, G. J., Biggar, H. H. and Trout, C. E., The rag-doll seed tester. Farmers' Bull. 948. U.S.D.A. (1918).
3) Conger, A. D., The cytogenetic effect of sonic energy applied simultaneously with X-rays. Proc. Nat. Acad. Sci. **34**, 470–474 (1948).
4) Gustafsson, A., The advantageous effect of deleterious mutations. Hereditas **33**, 573–575 (1947).
5) Harvey, E. W., Biological aspects of ultrasonic waves-A general survey. Biol. Bull. **59**, 306 (1930).
6) Hersh, A. H., Karrer E. and Loomis, A. L., An attempt to induce mutation in *Drosophila melanogaster* by means of supersonic vibrations. Amer. Nat. **64**, 552–559 (1930).
7) Istomina, O. and Ostrovsky, E., The effect of super-sonic vibrations on potato growth. Compt. rend. acad. sci. U.R.S.S. **1**, 635 (1935).
8) Istomina, O. and Ostrovsky, E., The effect of ultra-sonic vibrations on plant development. Compt. rend. acad. sci. U.R.S.S. **2**, 155–160 (1936).
9) Jones, D. F., Heterosis resulting from degenerative changes. Genetics **30**, 527–542 (1945).
10) Newcomer, E. H. and Wallace, R. H., Chromosomal and nuclear aberrations induced by ultrasonic vibrations. Am. J. Botan. **36**, 230–236 (1949).
11) Selman, G. G. and Wilkins, M. H. F., The production of high intensity ultrasonics at megacycle frequencies. J. Sci. Instruments **26**, 229–231 (1949).
12) Singleton, W. R., Hybrid sweet corn. Bull. 518 Conn. A.E.S. (1948).
13) Stadler, L. J., Some genetic effects of X-rays in plants. J. Heredity **21**, 2–19 (1930).
14) Tatum, L. A. and Zuber, M. S., Germination of maize under adverse conditions. J. Am. Soc. Agron. **35**, 48–59 (1943).
15) Wallace, R. H., Bushnell, R. J. and Newcomer, E. H., The induction of cytogenetic variation by ultrasonic waves. Science **107**, 577 (1948).

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STUDIES IN THE RESISTANCE OF CERTAIN VARIETIES OF BANANA TO PANAMA DISEASE

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PART I
INTERNAL FACTORS FOR RESISTANCE AND ANTIBIOTICS

Introduction. Panama disease of bananas is caused by a fungus, *Fusarium oxysporum-cubense,* which normally enters the banana plant through the root bases, grows through the rhizome or sucker ('bulb') to the pseudostem and leaf bases, causing a progressive wilting of the leaves in acropetal succession and finally death of the plant. W a r d l a w has investigated the course of infection of the highly susceptible Gros Michel variety of banana by the Panama disease organism, and has emphasised that "the disease is soil-borne and infection always proceeds from the roots and rhizomes upwards to the pseudostem and leaves" [10]). Attempts to innoculate even the very susceptible Gros Michel through the petioles and pseudostems have been unsuccessful. After the fungus has penetrated into the root bases there seems to be no further barrier to invasion and the fungus grows through the sucker or 'bulb' into the pseudostem and to the petioles [10]).

The cause of resistance in certain varieties must therefore be looked for in the roots and rhizomes. In this first paper those experiments are reported which were designed to test the hypothesis that an internal factor conferring disease resistance is present in resistant varieties of banana.

M a r t i n [5]) has reviewed much of the literature on the nature of resistance to soil borne pathogens. B r o w n, B r o o k s and B a w d e n [2]) discuss the causes of the resistance shown by plants to facultative, obligate and virus parasites respectively. Resistance has been claimed to be due to the following characters.

(*a*) Rate of cork formation. E.g. resistance of potato varieties to common scab, *Actinomyces scabies*, and Powdery scab, *Spongospora subterranea*, is claimed to be correlated with rapid rates of isolation of the pathogen by cork formation [6]). T i s d a l e has claimed that resistance of flax varieties to *Fusarium lini* is also correlated with the rate of cork formation [9]).

(*b*) Toxic substances produced by the plant. It has been demonstrated that resistant varieties of plants frequently contain a substance toxic to the pathogen, which is absent from the susceptible varieties or present in smaller amounts. R e y n o l d s [7]) showed that the HCN containing glycoside, linamarin, was present in greater amounts in flax plants resistant to *Fusarium lini* than in the susceptible varieties and that *Fusarium lini* is extremely sensitive to HCN poisoning. He also found other toxic substances present in the resistant plants. A n g e l l, W a l k e r and L i n k [1]) found protocatechuic acid in the outer scales of onion varieties resistant to onion smudge, *Colletotrichum circinans*. I r v i n g [3]) has isolated a fungicidal substance 'tomatin' from tomato plants, and has shown that this substance disappears from susceptible varieties of tomato on infection by *Fusarium oxysporum-lycopersici* but remains present in the resistant varieties. R u b i n, A r t s i k h o v s k a y a and P r o s k u r n i k o v a [8]) have claimed that resistance of potatoes to infection by *Phytophthora infestans* is determined at least in part by oxidative changes in the contained phenols.

(*c*) It has been suggested that susceptibility may be due to the presence of an internal chemical factor in the susceptible varieties which is essential or stimulant to the pathogen, but which is absent or in smaller amount in the resistant varieties [4]).

It seemed of interest to determine whether any of these three forms of resistance could be found in banana varieties which are known to be immune to Panama disease.

W a r d l a w has shown that infection of bananas by the Panama disease organism does not take place through wound surfaces on the very susceptible Gros Michel banana, but that infection is only through active young roots. It seemed, therefore, unlikely that the rate of cork formation could be the effective means by which immune varieties of banana are protected from infection. The experiments to be described were, therefore, limited to tests for the pre-

sence of toxic substances in immune banana varieties, and for the presence of growth stimulating substances and necessary nutrients for the fungus in susceptible varieties.

In these experiments several varieties of banana were used, in particular the variety Gros Michel, which is economically very important and very susceptible to Panama disease, and the genetically allied variety, Congo, which is immune to Panama disease. The varieties used are classified below according to cytology and disease resistance.

All are parthenocarpic representatives of *Eumusa* spp., *M. acuminata Colla* or the interspecific hybrid *M. acuminata Colla* × *M. Balbisiana Colla*.

TABLE I

Putative Parentage	Musa acuminata Colla	Musa Balbisiana Colla × Musa acuminata Colla
Susceptible to Panama disease (S)	Gros Michel (3 *x*)	Silk Fig (3 *x*)
Immune to Panama disease or virtually so (I)	Congo (3 *x*)	Guindy (2 *x*)
	I.C. 1 (4 *x*)	
	I.C. 2 (4 *x*)	
Ploidy.	2 *x* (2*n* = 22)	
	3 *x* (2*n* = 33)	
	4 *x* (2*n* = 44)	

Experimental

(1) *The growth of Fusarium oxysporum-cubense on banana rhizomes.* Suckers of Gros Michel and Congo bananas were taken from field plots at Imperial College of Tropical Agriculture and were washed, first with water then with soap and water, and a layer of the cortex about 1 cm thick was then removed with a flamed scalpel. The surface was again washed with tap water and the rhizome was soaked in a 1% solution of Aretan (soluble organo-mercuric fungicide) for 5 minutes. It was then washed thoroughly in several successive changes of sterile distilled water, and a further 1 cm layer was cut off from the whole surface with a flamed scalpel. The rhizome was then flooded with absolute alcohol, of which excess was drained off and the remainder was rapidly burned off. The surface of the sucker was removed once more, and was then cut into slices 0.5 cm thick from which discs were cut with a 1.0 cm

sterile cork borer. These discs showed no signs of contamination when left for 10 days in a damp atmosphere in sterile Petri dishes. Fresh discs prepared as described were streaked with a spore suspension of *Fusarium oxysporum-cubense*. Rapid growth of the fungus was obtained on discs of both Congo and Gros Michel rhizome. There was no difference in the rate of spread of the fungus or in spore production on the discs of the two varieties.

Similarly prepared discs of Congo and Gros Michel were placed in Petri dishes in 1 cm holes cut with a sterile cork borer in a glucose-Marmite-peptone agar medium previously seeded with *Fusarium oxysporum-cubense* spores. Uniform growth occurred over all the medium and spread over the discs of both varieties. There was no stimulation or depression of growth in the medium by either variety of banana.

(2) *The growth of Fusarium oxysporum-cubense on agar media prepared from banana rhizomes.* Agar media were prepared from the rhizomes of Gros Michel and Congo, Silk Fig and Guindy. The outer cortex was cut away from selected banana rhizomes. The remainder was cut into small cubes and ground in a Waring blender with twice the weight of distilled water. The pulped product was squeezed through muslin and the extract was centrifuged at 25,000 r.p.m. in a Sharples vertical centrifuge — repassing the extract until a clear liquid was obtained. After standing at room temperature for 12 hours, the extract was doubled in volume by addition of distilled water.

Agar was added to 2½% weight/volume and the medium was steamed for one hour. The pH was adjusted to 6.8 and the medium was autoclaved at 15 lbs pressure for half an hour. If thermolabile substances were present they would of course be destroyed in this process. The extracts were characteristically coloured when prepared as media. The media differed in the time taken to remelt in a water bath at 100°C, the media from the resistant varieties taking twice as long to melt as those prepared from the susceptible varieties, (Table II).

The media were poured into sterile Petri dishes and holes of 1 cm diameter were cut in the medium with a cork borer. Into these holes were placed discs of 1 cm diameter, of glucose-Marmite-peptone agar on which *Fusarium oxysporum cubense* was growing

TABLE II

The Colour and melting properties of agar media prepared from banana rhizome extracts

Supposed Parentage	Colour of medium	Melting properties	
		Melt rapidly	Melt slowly
Musa acuminata	Grey	Gros Michel (S)	Congo (I)
Musa acuminata × Musa Balbisiana	Red	Silk Fig (S)	Guindy (I)

rapidly. The agar was also innoculated with spores of *Fusarium oxysporum-cubense* by pin-point innoculation. The growth rate of the fungus was measured as rate of increase in diameter of the developing colonies. The growth rate was the same on all media. The rhizomes of the bananas prepared in this way did not differ with respect to any growth stimulating or growth inhibiting substances.

(3) *The effects of extracts of banana rhizomes on the growth of microorganisms, especially Fusarium oxysporum-cubense.* (*a*) Water extracts. Rhizomes of Congo and Gros Michel were macerated with equal weights of distilled water in a Waring blender and extracts were made by squeezing through muslin. Half of each extract was boiled for two minutes to effect partial sterilisation, and both the heated and unheated halves of each extract were tested for the presence of substances toxic to *Fusarium oxysporum-cubense* by placing 0.4 ml of the extract in a hole cut with a 1 cm cork borer in glucose-Marmite-peptone agar heavily seeded with spores of *Fusarium o.-c.* (20 ml of medium in each 9 cm diam. Petri dish).

There was no indication of inhibition of the fungus growth by any of the heated extracts although about half of the untreated Congo extracts produced rings of inhibition around the holes. A bacterium strongly antagonistic to *Fusarium oxysporum-cubense* was isolated from the Congo extracts and was considered to be responsible for the antifungal activity of the Congo extracts. It is interesting that this bacterium was only present in the Congo extracts. The distribution and significance of fungistatic organisms will be considered in the second part of this paper.

In a further experiment, extracts were completely sterilised. An extract of the rhizome sap from Congo and Gros Michel was

TABLE III

Antibiotic spectra of banana root extracts
Inhibition zone width

Micro-organism	*B. subtilis*	*B. solanacearum*	*Fusarium*	*Myc. phlei*
BANANA		*1st DAY*		
Gros Michel	5.5	4.5	1.2	2.5
	5.2	4.5	1.0	2.5
Congo	7.0	5.5	2.0	5.0
	7.5	5.5	1.0	9.5
Silk Fig	4.0	3.7	3.0	0.0
	3.5	2.5	2.5	0.0
Guindy	3.5	0.0	3.0	0.0
	4.0	0.0	4.5	0.0
I.C. 1	4.0	1.5	5.0	2.0
	4.2	2.0	5.0	2.5
I.C. 2	2.0	0.0	1.7	0.0
	4.0	0.0	1.5	0.0
BANANA		*3rd DAY*		
Gros Michel	3.5	3.2	0.0	2.2
	3.5	3.5	0.0	1.7
Congo	5.5	4.0	0.0	4.5
	6.0	4.0	0.0	4.5
Silk Fig	2.0	1.5	0.0	0.0
	2.0	2.0	0.0	0.0
Guindy	1.5	0.0	0.0	0.0
	2.2	0.0	0.0	0.0
I.C. 1	2.0	1.5	0.0	2.0
	2.5	1.0	0.0	2.5
I.C. 2	1.0	0.0	0.0	0.0
	1.5	0.0	0.0	0.0

made by freezing 73 g of rhizome and macerating in a Waring blender after thawing, with 73 ml distilled water. The extract was then cleared by centrifuging at 3500 r.p.m. and sterilised with chloroform for 4 hours. The extracts thus obtained were sterile and gave no inhibition of *Fusarium oxysporum-cubense* on Glucose-Mamrite-peptone agar.

(*b*) Alcoholic extracts. Roots of all the varieties listed in Table I were examined for antifungal and antibacterial substances in alcoholic extracts. Extracts were prepared as follows. 44 g of roots were macerated in a Waring blender in 200 ml of 95% alcohol. The mixture was allowed to extract over night. The mixture

was then filtered to separate the pulp from the extract and the liquid was evaporated to 1 ml at reduced pressure in a water bath at temperature 60–80°C. Three millilitres of methyl alcohol were then added to keep the extracts sterile. On the following day the extracts were again evaporated down to remove the methyl alcohol and the extracts were made up to 10 ml with distilled water. These extracts were tested against *Bacillus subtilis* and *Fusarium oxysporum-cubense* by the method described for water extracts. They did not inhibit the growth of either organism.

The extracts were again evaporated to 2 ml and tested against *B. subtilis*, *B. solanacearum* (isolated from *Solanum tuberosum* from American type culture collection), *Mycobacterium phlei* and *Fusarium oxysporum-cubense*.

This time considerable rings of inhibition were observed. The following results were obtained. See Table III.

Inhibition of all organisms by all extracts was observed on the first day after plating except Guindy and I.C. 2 against *B. solanacearum* and Silk Fig, Guindy and I.C. 2 against *Mycobacterium phlei*. On the third day the *Fusarium oxysporum-cubense* plates showed no inhibition, the fungus having grown over the first day inhibition rings.

The Significance of change in Ring diameter. In a number of cases in which banana root extracts have been plated out onto media containing a test organism, there is a wide inhibition zone on the first day and a decrease on subsequent days. To a great extent this seems to be characteristic of the organism under test rather than of the test solution.

For example, on the first day after plating *Fusarium oxysporum-cubense* and testing the series of banana root extracts against it, inhibition rings were obtained. On the third day, there was heavy overgrowth of the inhibition rings and by the fourth day after plating there was no significant ring left. Possible explanations for this phenomenon are — (*a*) The organism slowly inactivates the antibiotic: (*b*) The antibiotic substance is unstable or volatile: (*c*) There are present, in the culture, forms which are not inactivated by or become adapted to the antibiotic and which spread and become apparent in the cleared zone, becoming apparent late because they are present in a very small proportion of the population. It is very significant that the colonies which appear in the inhibition zone are usually discrete and separate from the main development of the organism on the rest of the plate. This suggests that the growth on the cleared zone is from isolated spores and is not overgrowth from the organism outside the inhibition ring. There is a clear difference between the behaviour of *B. subtilis* and *B. so-*

lanacearum in respect to change in ring size. *B. subtilis* shows rapid overgrowth of the rings, this growth taking place from discrete colonies in the inhibition ring, but there is no change in ring diameter with *B. solanacearum*.

There is evidence from these results that more than one antibiotic substance can be extracted from the roots of banana.

All the extracts were active against *B. subtilis* and the activities, although not the same for the varieties, run parallel for each variety tested. There is therefore evidence that a substance, or substances, which can be called A, is present in all the banana roots. A further substance active against *B. solanacearum* is present in the roots of Gros Michel, Congo, Silk Fig. and I.C. 1, which is absent from the remainder; this can be called B. A further substance active against *Mycobacterium phlei* is present in the roots of Gros Michel, Congo and I.C. 1; this substance can be called C. The antibiotic spectra of the varieties tested were therefore:

Gros Michel	A. B. C.	Congo	A. B. C.
Silk Fig	A. B.	I.C. 1	A. B. C.
Guindy	A.	I.C. 2	A.

Although these alcoholic extracts of roots gave inhibition of *Fusarium oxysporum-cubense*, there is no complete correlation between the presence of the antibiotic and resistance. Congo$^{(I)}$ showed more activity than Gros Michel$^{(S)}$, Guindy$^{(I)}$ showed a little more than Silk$^{(S)}$, but Silk$^{(S)}$ gave a higher activity than Congo$^{(I)}$, and I.C. 2$^{(I)}$, a good resistant strain contained less than any of the susceptible varieties. The extraction by alcohol of a substance, which is not extracted by water, may be evidence that it is a product of the treatment rather than present in living tissues. This is also suggested by the absence of toxicity on first evaporation of the alcoholic extract.

Alcoholic extracts of the rhizomes of banana varieties prepared as described above for roots, had no inhibiting effect on *Fusarium oxysporum-cubense*, and showed little varietal difference in antibiotic activity against *B. subtilis*, *B. solanacearum* and *Mycobacterium phlei*.

Summary and Conclusions

There is no evidence that there is present in the roots or rhizomes of bananas any stable substance which prevents the growth of the Panama disease organism. Discs of living tissue and media prepared from rhizomes of resistant and susceptible varieties of banana support free and equal growth of the pathogen. Alcoholic extracts of banana roots show antibiotic activity against *Bacillus subtilis, Bacterium solanacearum, Mycobacterium phlei* and ephemeral activity against *Fusarium oxysporum-cubense*. There are varietal differences in the quantity and type of antibiotic produced, but these show no correlation with disease resistance.

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BIBLIOGRAPHY

1) Angell, H. R., Walker, J. C. and Link, K. P., The relation of protocatechuic acid to disease resistance in the onion. Phytopathology, **20**, 431–438, (1930).
2) Brown, W., Brooks, F. T., and Bawden, F. C., A discussion on the physiology of disease resistance in plants. Proc. Roy. Soc. London B. **135**, 171–195, (1948).
3) Irving, G. W. Jr., The significance of tomatin in plant and animal diseases. J. Wash. Acad. Sci., **37**, 293–296. (1947).
4) Leach, J. G., The parasitism of *Puccinia graminis tritici* Erikss. and Henn. and *Puccinia graminis compacti* Stak. and Piem. Phytopathology, **9**, 59–88 (1919).
5) Martin, H., The Scientific Principles of Plant Protection. E. Arnold & Co., 3rd Edn. (reprinted 1948).
6) Min. of Agriculture Leaflet No. **137** (1925).
7) Reynolds, E. S., Some relations of *Fusarium lini* and potassium cyanide. Am. J. Botany **11**, 215–217, (1924).
Reynolds, E. S., Studies on the physiology of Plant Diseases. Ann. Missouri Botan. Garden, **18**, 57–95, (1931).
8) Rubin B. A., Artsikhovskaya E. V. and Proskurnikova, T. A., Oxidative changes of phenols and their role in the resistance of potatoes against *Phytophthora infestans*. Biokhimiya, **12**, 141–152 (1947).
9) Tisdale, W. H., Flaxwilt: a study of the nature and inheritance of wilt resistance. J. Agr. Research **11**, 573–605 (1917).
10) Wardlaw, C. W., Diseases of the banana. Macmillan & Co. (1935).

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PART II. THE RHIZOSPHERE

Introduction. There have recently been many demonstrations of the presence in soils, of micro-organisms which are capable of antagonising plant pathogens in culture. For example, Meredith [5]) has shown that there are present in banana soils many actinomycetes which are antagonistic to *Fusarium oxysporum-cubense* in culture. He found however, no correlation between the resistance of soil type to the development of the disease and the abundance of antagonists. In a few cases it has been demonstrated that the introduction of an antagonist to a soil, sterile except for the presence of a plant pathogen, has depressed the incidence of the disease in plants subsequently grown in that soil. Weindling [7]) and Brian [1]) have reviewed much of the work on microbial antagonism to plant pathogens. The greatest claims for the significance of microbial antagonists to soil borne pathogens have been made by Russian workers, notably Novogrudskij [6]) who states that the 'bacterisation' of seeds of linseed, with cultures of organisms which have been shown to lyse *Fusarium lini* in culture, greatly reduces the incidence of disease in the developing seedlings.

The establishment of a root disease in a crop is frequently dependent on soil conditions. This subject has been reviewed by Garrett [3]). Frequently the addition of organic matter to soils heavily infected with some root disease organism, has resulted in a depression of the incidence of the disease, supposedly because of the stimulation of soil saprophytes which are antagonistic to the pathogens. The growing plant is itself responsible for making very

considerable additions of organic matter to the soil, in the form of decaying root cap cells, root hairs and the cortex of older roots, together with possible root excretions from living cells. The population of micro-organisms on the root surfaces is therefore expected to resemble that of an organically manured soil. L o c h h e a d and his associates have shown this to be true and have made an intensive study of the effects of growing plants in modifying the soil microflora. They have shown these effects to be both qualitative and quantitative [4]).

Not only is the soil microflora changed by the presence of plant root systems, but different plants may influence the soil population in different ways. The effect of this change on soil borne pathogens has been shown by D e y [2]) who has shown that the intercropping of peas with Sorghum, reduced the incidence of *Fusarium udum* in the peas. K a t z n e l s o n *et al.* [4]) review their own work on the differences between the rhizosphere flora of resistant and susceptible varieties of flax to the disease caused by *Fusarium lini*. In these cases the resistant varieties support a smaller microbial population in their rhizosphere than the susceptible varieties.

The experiments to be described, were designed to detect differences between the rhizosphere flora of banana varieties and to discover to what extent the differences which were found were related to the nutrient content of the underground parts of the banana varieties tested. For these experiments, banana varieties immune and susceptible to Panama disease were chosen to test the hypothesis that there is a correlation between disease resistance and the nature of the rhizosphere population. The organisms isolated from the rhizospheres were also tested for the production of antibiotics to *Fusarium oxysporum-cubense* in culture.

Experimental

(1) *The rhizosphere flora of banana varieties.* Two suckers were taken from each of the following banana varieties in the field.

Gros Michel	Silk Fig	(Both susceptible to Panama disease).
Congo	Guindy	(Both immune to Panama disease).

(See Part I, Table I for further details of these varieties).

The suckers were cleaned of attached soil and the roots were removed. The suckers were then planted in individual 10″ pots con-

taining a loamy soil. No bananas were planted in two control pots.

After one month, active growth of new roots had occurred and from each pot a 10 g sample of roots plus attached soil was added to one litre of sterile Ringer's solution at one tenth of normal strength. The flasks were shaken in a mechanical shaker for ten minutes. From each flask, 1 ml of suspension was taken into 99 ml of sterile Ringer/10 to give a dilution of 1 part of the washings of 1 g of root sample to 10,000 parts of diluent. After shaking, a further dilution was made by taking 1 ml of the above dilution to 99 ml of sterile Ringer/10 to give a final dilution of 1 part in 1,000,000. From this final dilution, samples of 2.0 ml, 0.6 ml and 0.2 ml were pipetted into tubes containing 20 ml of molten soil extract agar at 45°C and after rotation of the tubes to mix the inoculum with the agar the mixture was poured into sterile Petri dishes. In all, ten dilution series were prepared, two for each banana variety and two for the control soil. Three levels of plating density were obtained by using the three sizes of sample from the 1/1,000,000 dilution and each of these ultimate samples was replicated three times.

The soil extract agar medium used in this experiment was prepared by a modification of Löhnis method as follows. 1 kilogram of soil was autoclaved with 1 litre of distilled water for ½ hour at 1½ atmospheres pressure. The resultant turbid suspension was passed through a Sharple's vertical centrifuge until a clear extract was obtained. The original method of Löhnis which involved filtration and refiltration through filter paper to clear the liquid was slow, sometimes taking as long as two to three days to obtain 1 litre of extract. The centrifuge method reduces this period to about twenty minutes. The filtrate was made up to 1 litre with distilled water and 0.5 g of K_2HPO_4 was added with 20 g of agar. After steaming to dissolve the agar, the pH was adjusted to 6.8 and the medium was tubed in 20 ml aliquots and autoclaved for ½ hour at 1½ atmospheres.

The roots from which the rhizosphere samples were taken were weighed, after drying at 100°C to constant weight. The colonies which developed on the agar plates were counted, see Table I. The plates innoculated with 2.0 ml were discarded because the populations were too high for accurate counts to be made.

Discussion of results. The expression of bacterial counts from rhizosphere samples presents great difficulty. Lochhead and his co-workers have expressed their results in terms

TABLE I

Determination of banana rhizosphere population

Variety of banana	Volume of 1/1.000.000 dilution per plate					Total difference of observations from mean soil count for each variety $(2) - \bar{X} + (3) - \bar{Y}$	Weight of Root = w	Estimated total bacteria per dry root $\frac{(2) - \bar{x} + (3) - \bar{y}}{4.8 \times 0.00,0001 \times w}$
	(1) 2.0 ml	(2) 0.6 ml	$(2)-\bar{x}$	(3) 0.2 ml	$(3)-\bar{y}$			
Gros Michel replication add sampling A 1	—	33	(17)	15	(8)	220	0. 327 g	140,163,000
Gros Michel replication add sampling A 2	—	51	(35)	11	(4)			
Gros Michel replication add sampling A 3	—	55	(39)	18	(11)			
Gros Michel replication add sampling B 1	—	42	(26)	22	(15)			
Gros Michel replication add sampling B 2	—	36	(20)	21	(14)			
Gros Michel replication add sampling B 3	—	37	(20)	18	(11)			
Congo 1	127	33	(17)	11	(4)	105	0. 180 g	121,458,000
Congo A 2	130	25	(9)	10	(3)			
Congo 3	123	27	(11)	13	(6)			
Congo 1	61	20	(4)	8	(1)			
Congo B 2	86	46	(30)	6	(−1)			
Congo 3	75	32	(16)	12	(5)			
Silk Fig 1	89	21	(5)	5	(−2)	39	0.102 g	79,538,000
Silk Fig A 2	71	16	(0)	9	(2)			
Silk Fig 3	75	26	(10)	7	(0)			
Silk Fig 1	49	28	(12)	4	(−3)			
Silk Fig B 2	71	26	(10)	7	(0)			
Silk Fig 3	75	26	(10)	7	(0)			
Guindy 1	83	21	(5)	9	(2)	108	0.315 g	71,250,000
Guindy A 2	81	17	(1)	4	(−3)			
Guindy 3	81	13	(−3)	9	(2)			
Guindy 1	122	52	(36)	6	(−1)			
Guindy B 2	111	34	(18)	21	(14)			
Guindy 3	131	45	(29)	15	(8)			
Soil 1	86	8		3				
Soil A 2	56	12		8				
Soil 3	68	14	$x = 16$	7	$y = 7$			
Soil 1	62	14		11				
Soil B 2	61	29		6				

of the number of bacteria and other organisms per gram of soil washed off the root system of a plant which has been pulled from the soil and shaken. This method suffers from the disadvantage that the amount of soil which remains attached to a root system which has been shaken, is determined by the stickiness of the soil, varying with soil type and moisture content. Ideally, the expression of rhizosphere effect should take into account the amount of root present and perhaps also the area of root surface, which in most cases is extremely difficult to measure. In the final column of Table I the number of organisms developing per plate has been summed for each variety and for the control soils. The count obtained from the control soil has been subtracted from the rhizosphere counts, and the difference has been corrected to give the total estimated difference between the count from 10 g of control soil and that from 10 g of root plus attached soil. This has then been divided by the dry weight of roots. The order of the results is not of course changed by the subtraction of the control soil count, although the differences would be somewhat reduced if this was not done. In this form, the figures in the final column of Table I represent the increase in numbers of micro-organisms brought about by the presence of 1 g dry weight of roots in a soil sample. There is a very clear difference between the numbers of organisms supported by the root systems of the genetically related pair, Congo and Gros Michel and the Silk Fig and Guindy pair. In each pair it is noteworthy that the resistant variety supports a smaller rhizosphere flora than the susceptible, but in the absence of replication it is impossible to judge the significance of the differences found. The counts obtained however, support the view that resistant varieties bear a smaller rhizosphere flora than susceptible, which L o c h h e a d and his associates had suggested from experiments with flax and tobacco.

(2) *The interaction between the rhizosphere flora of banana varieties and their growth on media prepared from these varieties.* Agar media were prepared from the rhizomes of Congo, Gros Michel, Silk Fig and Guindy bananas as described in Part I of this paper. Soil extract medium was prepared as previously described and a synthetic medium, glucose-Marmite-peptone agar was made.

TABLE II

Plate Counts on Medium/Root sample interaction experiment

Samples of soil and root microfloras	ml of 1 : 1,000,000 Dilution/ Plate	MEDIA							
		Gros Michel	Congo	Silk	Guindy	Soil extract	Glucose-Marmite peptone-agar	Dilution Totals	Totals
Gros Michel .	2.0	37	55	18	12	54	82	258	
	0.6	11	23	7	3	22	43	109	407
	0.2	5	13	2	2	5	13	40	
Congo	2.0	233	222	44	20	164	268	951	
	0.6	32	35	15	9	53	109	253	1301
	0.2	8	19	5	2	24	39	97	
Silk	2.0	80	118	57	32	80	185	552	
	0.6	10	24	5	2	17	50	108	742
	0.2	9	13	8	0	7	45	82	
Soil A	2.0	40	87	10	3	93	108	341	
	0.6	8	12	7	2	18	77	124	506
	0.2	1	4	3	1	15	17	41	
Soil B	2.0	34	26	15	19	30	65	189	
	0.6	4	5	1	0	17	35	62	259
	0.2	1	3	1	0	2	1	8	
Total . . .		513	659	198	107	601	1140	3215	3215

Glucose-Marmite-Peptone agar:

To 1 litre of distilled water was added 10 g of glucose, 5 g of peptone and 3 g of Marmite; after boiling the mixture, 20 g of agar was added and stirred until dissolved. The pH was then adjusted to 6.8 and the medium was tubed in 20 ml aliquots and autoclaved for ½ hour at 1½ atmospheres pressure.

Roots of Gros Michel, Congo and Silk Fig bananas were sampled in the field. Two soil samples, A and B were taken from midway between a row of Congo and a row of Gros Michel bananas. The soil samples were well out of the influence of the banana roots, but were under a mixed grass vegetation; no bare areas were available for sampling.

In the laboratory, 10 g subsamples of each of the root samples were taken — these were composed of root plus the soil particles which remained attached to the roots after shaking. 10 g subsamples of soil were taken from samples A and B.

Dilutions were prepared in the same way as in Experiment I. 2.0 ml, 0.6 ml, and 0.2 ml aliquots were taken from the 1/1,000,000 dilutions of each soil and root sub-sample, onto each type of agar medium. After seven days incubation at 25°C the colonies which had developed on the plates were counted and the counts obtained are presented in Table II.

Discussion of results. The results presented in Table II have been analysed in several ways. An analysis of variance of the logarithms of the plate counts in Table II is given in Table III.

TABLE III

Analysis of variance of Medium/Root Sample interaction experiment
(Analysis of the logs of values in Table II)

Main effects	Sum of squares	D.F.	Variance	Variance ratio	Probability
(Variety of root sampled	5.70	4	1.425	35.09	less than .001)
Type of medium	12.22	5	2.444	60.19	*less than .001*
Dilution	16.08	2	8.041	198.05	less than .001
1st order interaction					
Variety/Medium. . . .	1.053	20	0.0526	1.295	—
Variety/Dilution . . .	0.628	8	0.0785	1.933	.05 — 0.1 *)
Medium/Dilution . . .	0.755	10	0.0755	1.859	.05 — 0.1 *)
2nd order interaction					
Variety/Medium/Dilution = Error	1.626	40	0.0406		
Total	38.062	89			

*) To be discussed in a later publication.

There is significant difference between the media used, in their power of supporting the microbial populations introduced from the soil samples. The statistically significant difference between the total numbers of the rhizosphere samples should be ignored as no correction was made in this experiment to express rhizosphere counts in terms of the amount of root present in each sample. However it is possible to compare the rhizosphere floras of the banana varieties with respect to their relative development on the various media. In Table IV the results presented in Table II have been transformed,

TABLE IV

The development of soil and banana root populations of micro-organisms on media derived from banana extracts, as percentages of the development on soil extract agar

Root and soil microflora samples	Root and soil media				
	Gros Michel	Congo	Silk	Guindy	Soil
Gros Michel . . .	65.4	112.3	33.3	20.9	100.0
Congo	113.2	114.5	26.5	12.4	100.0
Silk	95.2	149.0	67.3	32.7	100.0
Soil A	37.3	81.7	15.9	4.7	100.0
Soil B	79.6	69.3	34.7	38.7	100.0

by summing over dilutions and expressing the number of colonies developing on a banana extract agar as a percentage of the number of colonies developing from the same innoculum on soil extract agar. The results are also expressed in a histogram, Fig. 1. The differences between media show clearly, but the differences in reaction of rhizospheres to the various media are particularly interesting. The soil extract agar is a better nutrient medium for the soil organisms than any rhizome extract, but the rhizosphere organisms are more completely represented on Congo. Silk Fig and Guindy media are very much poorer in their power of supporting a mixed microbial population than Congo and Gros Michel, and this is to be expected from a consideration of the magnitude of the microbial populations which these varieties support in the field (Expt. 1).

It is clear from this experiment, that banana varieties differ in the nutrient content of their underground parts and it seems likely that it is this difference which determines the differences between the microbial populations which the varieties support on their roots in the field.

(3) *The presence of organisms antagonistic to Fusarium oxysporum-cubense in the rhizosphere of bananas.* After counting the colonies obtained in experiment (2) the plates were flooded with glucose-Marmite-peptone agar molten at 45°C which had been heavily seeded with spores of *Fusarium oxysporum-cubense*. Occasional actinomycetes showed antagonism to the development of the fungus,

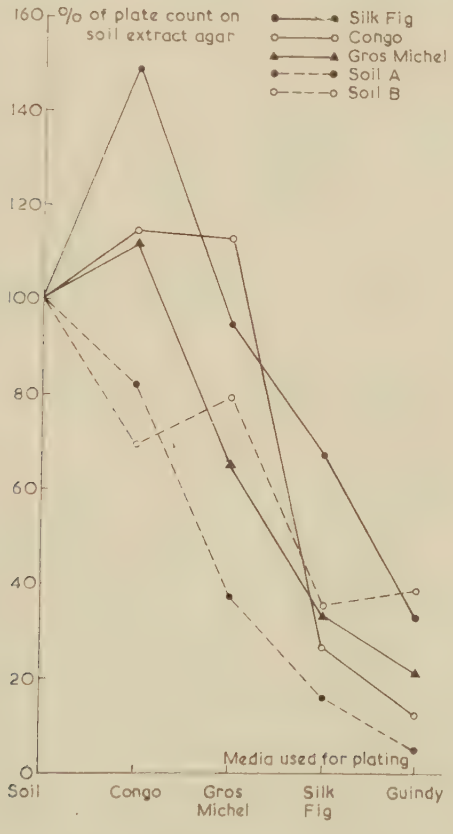

Fig. 1

demonstrated by rings of inhibition of the fungus in the agar above the colonies, but there was no correlation between the variety of banana and the abundance of these organisms.

A further test was made for the presence of antagonists to the fungus. Dilutions of the root washings of Congo and Gros Michel bananas were plated onto soil extract agar. After seven days, from

each of half the plates, 20 bacterial colonies and from the other half 20 actinomycete colonies were isolated and inoculated into tubes of sterile 1% Difco yeast extract broth. After ten days the broth was tested for antibiotic activity to *Fusarium oxysporum-cubense*. Each tube of extract was boiled for two minutes to effect partial sterilisation and 0.4 ml was added from a microdropper into a hole cut with a 1 cm cork borer in agar heavily seeded with spores of the fungus in Petri dishes. The results are given in Table V. A high pro-

TABLE V

Bacterial antagonists isolated from soil plates of the rhizospheres of Congo and Gros Michel bananas

Banana variety	Plate Count	Antagonists in 20 isolated	Percent Antagonists
A, B,	C, = replicate root systems sampled		
a, b,	c, d, = replicate plates made from each sample		
Gros Michel			
root sample . . . A (a)	186	1	5.0
(b)	49	0	0.0
B (a)	39	0	0.0
Congo			
root sample . . . A (a)	35	11	55.0
(b)	138	6	30.0
(c)	98	4	20.0
[(d)	303	0	0.0]
B (a)	43	2	10.0
[(b)	224	0	0.0]
(c)	215	3	15.0
C (a)	54	5	25.0

portion of bacteria isolated from the roots of Congo showed activity but only one antagonist was isolated from Gros Michel. At high plating densities the antagonist seemed to be suppressed and there was a sharp fall off in the percentage of antagonists obtained from any one root sample when the plating density was higher than 200 colonies per plate. (This plating density effect will be considered in detail in a later paper).

The 32 isolations of the antagonist were compared and from preliminary examination of growth rate, morphology, colony form and antibiotic production no difference was found between the isolations.

Further investigations of this organism are being made at the Colonial Microbiological Research Institute, Port of Spain, Trinidad.

The bacterium failed to produce antibiotics from subsurface colonies although its surface colonies were extremely active against *Fusarium oxysporum-cubense*.

Dr. A. C. Thaysen informs me that his investigations of this organism show that it requires fully aerobic conditions for antibiotic formation, which would account for the above observation.

Discussion of results. Further investigations are needed to determine whether the abundance of fungistatic organisms on the roots of the Panama disease immune Congo banana and its scarcity on Gros Michel roots was an ephemeral manifestation of the general difference in microflora between the two varieties or whether it is a permanent character of the varieties.

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Summary

The rhizosphere flora of banana varieties differs quantitatively from variety to variety. The comparison of rhizospheres is difficult, because the form of expression of the results may take many forms, none of them completely satisfactory. In the method adopted here, the two genetic pairs of banana, Gros Michel — Congo and Silk Fig — Guindy differ widely in the rhizosphere flora which they support, the former pair supporting the greater

number of micro-organisms. Within each pair, the higher number is found on the variety susceptible to Panama disease. The nutrient requirements of rhizosphere floras are related to the sap of the banana variety from which they have been derived.

A bacterium strongly antagonistic to *Fusarium oxysporum-cubense* was isolated in high numbers from the rhizosphere of the Panama disease immune Congo banana, although this was virtually absent from the susceptible Gros Michel.

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BIBLIOGRAPHY

1. Brian, P. W., The production of antibiotics by micro-organisms in relation to biological equilibria in soil, S.E.B. Symposium No. 3, 357–372, Cambridge (1949).
2. Dey, P. K., Plant Pathology, Adm. Rep. Agric. Dep. U.P. 39–42 (1946–7).
3. Garrett, S. D., Root Disease Fungi, Chronica Botanica Co., 1944.
4. Katznelson, H., Lochhead, A. G., and Timonin, M. I., Soil micro-organisms and the rhizosphere, Bot. Rev., **14**, 543–587 (1948).
5. Meredith, C. H., The antagonism of *Actinomyces* to *Fusarium oxysporum-cubense*, Phytopathology, **33**, 403 (1943).
Meredith, C. H., The antagonism of soil organisms to *Fusarium oxysporum-cubense*. Phyto-pathology, **34**, 426–429 (1944).
6. Novogrudskij, D. M., Use of micro-organisms in control of fungal diseases of cultivated plants, Buo. Acad. Sci. U.S.S.R., Biol. Ser. **1**, 277–293 (1936).
Novogrudskij, D. M., Antagonistic interrelations among microbes and biological methods of combating fungus diseases of cultivated plants, Adv. Mod. Biol. U.S.S.R., **5**, 509–536 (1936).
Novogrudskij, D. M., Berezova, E., Nakhimovskaya, M. and Perviakova, M., The influence of bacterisation of flax seed on the susceptibility of seedlings to infection with parasitic fungi, Compt. Rend. Acad. Sci. U.S.S.R. **14**, 385–388, (1937).
7. Weindling, R., Microbial antagonism and disease control, Soil Science, **61**, 23–30, (1946).

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SOIL AGGREGATE STABILITY AS AFFECTED BY STERILIZATION WITH ETHYLENE OXIDE AND HEAT *)

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(avec un résumé français)

The methodology of laboratory sterilization of soil is of interest when effects on soil aggregation, caused by soil microorganisms or various materials, are being studied. In such studies it is often necessary to provide sterile soil either as a basis of comparison or as a starting material.

So far it has been a common practice to sterilize soil by exposure to high temperatures in a steam autoclave. It can be expected [2]) that this treatment will fail to result in an ideally sterile soil, an ideally sterile soil being one that differs from a non-sterile soil solely in lack of viable organisms.

An as alternative to heat sterilization (but with no particular reference to aggregation studies) Roberts *et al* [6]), Dalton and Hurwitz [3]), and Allison [1]) recently called attention to the possibilities of using ethylene oxide vapor. A satisfactory degree of sterilization — satisfactory as far as reducing the number of living organisms was concerned — was claimed to be feasible by all the above authors.

Since in the literature no data could be found showing the effect of heat or ethylene oxide on soil aggregation, the present study was undertaken. While it was to be expected that heat sterilization was objectionable as a preparatory treatment, it was hoped that ethylene oxide would not have limitations.

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Procedures. Five different soil lots were used in the experiment. All were Clarion loams from the Agronomy Farm, at Ames, Iowa. One was taken from a plot in continuous corn, one from a plot in the corn phase, one from a plot in the oats phase and one from a plot in the meadow phase of a corn-oats-meadow rotation, and one from a site in continuous bluegrass, located in an unused part of a driveway. The sampled plots had been cropped in the indicated manner for 35 consecutive years and had received no other treatment than liming. At the time of sampling the soils were forced gently through a screen of 8 mm opening. They were subsequently airdried in the laboratory.

The aggregate distribution was determined on 25 g aliquots that were wetted with a fine spray of water and wet sieved according to standard Yoder technique [8]). The mean weight-diameter of the size distributions were determined as described by the author [7]). The mean weight-diameter is an average aggregate diameter based on the weight of the separates obtained as a result of wet sieving.

Five different treatments were given to the soil with regard to sterilization. The first treatment was absence of sterilization. The second, third, and fourth treatments were sterilization with ethylene oxide at respectively 5, 10 and 15 in. of mercury partial pressure. The fifth treatment was sterilization with heat in a steam autoclave at a pressure of 15 p.s.i. for 20 minutes. Treatment was effected in duplicate on soil lots of 60 g. Thus, four 25 g aliquots could be taken for analysis of every soil lot and every treatment.

The apparatus for sterilizing the soil was a pressure cooker of 9 liter capacity. The lid of the cooker was fitted with three brass petcoks. One petcock was connected with a vacuum pump, another one went to a Bourdon-type gauge, reading in in. of mercury, and the third, to an inlet funnel for transferring the liquid ethylene oxide into the vessel.

Five 60 g lots of air-dry soil, in Erlenmeyer flasks, were placed at one time in the vessel, which was then completely evacuated. Ethylene oxide was now admitted carefully through the funnel until the pressure in the vessel had reached 5, 10 or 15 in. of mercury. The soils were left exposed to the vapor overnight. Finally, the vessel was completely evacuated, the vacuum pump being kept running for about 30 minutes to remove vapors of ethylene oxide.

Four sets of 50 soil samples, each set consisting of the 5 soils, subjected to the 5 different methods of sterilization, in duplicate, were prepared. After preparation each of the four sets was used in a different experiment.

In the first experiment (I) one of the sets of 50 soil samples was analyzed for aggregates without further treatment, except that the soil which was sterilized in the autoclave was allowed to become air-dry, as it had become somewhat damp.

In the second experiment (II) the next set of 50 samples was wetted with a quantity of distilled water to bring the moisture content to moisture equivalent; the soil was then allowed to become air-dry again and was analyzed for aggregates. Bringing the moisture content of the soils up to moisture equivalent was done by distributing a known amount of water drop by drop over the soil using a pipette. Without disturbing the soil at all it was then allowed to become air-dry again. The method of wetting may not be the best one from the standpoint of the speed at which wetting is accomplished. Wetting by vacuum flooding or wetting with a fine spray is more gradual but is not practical in the course of experiments where a great number of samples have to be wetted with a small and definite amount of water.

In the third experiment (III) the 50 samples were wetted with sterile distilled water up to the moisture equivalent, were stored in an incubator at 37°C for two weeks, subsequently allowed to become air-dry and then analyzed for aggregates. The reason for incubating the soils in sterile condition was particularly to study possible deviations from the results of experiment II. In order to obviate microbiological activity in the untreated soil lots, a drop of toluene was added to each soil sample. Although this in itself might have caused some change in the soil, no such effect was found, as may be seen by comparing the two values for untreated soils in Experiment II and III in Table II.

In order to make sure that the samples were alle sterile they were plated at the end of the incubation period of two weeks on a yeast extract agar. These agar plates were incubated for four days at 37°C and any occurring growth was noted. It appeared that the soils treated with ethylene oxide at 10 and 15 in. of mercury pressure were sterile as measured by this test, but that some of the samples treated at 5 in. of mercury still showed a little growth. The

amount of growth was so small that it was considered of no importance in its effects on aggregation.

In the fourth experiment (IV) the 50 samples were wetted with distilled water up to the moisture equivalent, thereafter reinoculated with a suspension of fresh garden soil, then incubated for two weeks at 37°C, allowed to become air-dry and analyzed for aggregates. The soil suspension for reinoculation was prepared by suspending 10 g of fresh garden soil in 25 ml of water. One ml of this suspension was added to every soil sample. Although the control lots were not sterilized, they were also inoculated so as to obtain comparable conditions. The fourth experiment had the purpose of ascertaining modifications of the effects noted in experiment III, caused by the activity of soil microorganisms. Considerable after-effect on microbiological activity is known as result of heat sterilization and Clark[2]) has demonstrated after-effects of sterilization with ethylene oxide.

Experimental results and discussion of results

Experiment I. Soil Sterilized and Analyzed. The results of the aggregate analyses are condensed in tables of analysis of variance and in tables of average values. The data can be handled in this manner because results are expressed as single values, namely, mean weight-diameters.

TABLE I

Analysis of variance of values for mean weight-diameters in mm obtained in experiment I on the influence of sterilization on aggregation of five different soil lots

Source of variance	Degrees of freedom	Sum of squares	Mean square
Methods of sterilization	4	2.048	0.512 *)
Soil lots	4	24.119	6.03 *)
Methods × Soil lots	16	1.590	0.0993 *)
Error	25	0.427	0.0171
Total	49	28.184	—

*) Highly significant (1% probability).

The analysis of the variance of the data obtained in the first experiment is given in Table I. That the differences between the five soil lots proved to be highly significant was expected, because the soils were purposely selected to cover a wide range of variation in

aggregate distribution. It is of interest to note from the interaction component that the different treatments did not have the same effect on the different soil lots. From the average values for individual soil lots and treatments, here not presented, it appears that the largest effect is found for continuous corn and that the effect for continuous meadow was smallest.

TABLE II

Average values of mean weight-diameters in mm for different methods of sterilization and for different soil lots in experiments I, II, III and IV

Method of sterilization	Experiment			
	I	II	III	IV
None	1.043	1.273	1.167	1.202
Ethylene oxide 5 in. Hg	1.087	1.169	1.549 *)	1.266
Ethylene oxide 10 in. Hg	1.241 *)	1.249	1.601 *)	1.376
Ethylene oxide 15 in. Hg	1.350 *)	1.205	1.168	1.200
Heat	1.606 *)	1.260	1.758 *)	1.195
Soil lot	**Experiment**			
	I	II	III	IV
Continuous corn	0.617	0.584	0.635	0.583
Corn of c.o.m.	0.815	0.733	1.112	1.041
Oats of c.o.m.	1.064	0.995	1.614	1.350
Meadow of c.o.m.	1.244	1.117	1.014	0.639
Continuous meadow	2.587	2.727	2.868	2.623

*) Highly significantly different from check (1% probability).

In Table II the average values for the five sterilization treatments are listed. As a whole, the effect of the treatments is highly significant. From the error component of Table I it was calculated that the least significant difference between any two averages was 0.168 mm at the 1% level and 0.118 mm at the 5% level. The significance of the differences, as indicated in Table II, refers to the deviation of the respective average value from the average value of the untreated soils.

The author does not attempt to explain the effects that were noted. This would be beyond the scope of this paper and would require more detailed studies. It deserves mentioning, however, that the possible effect of absorbed gases, as indicated by Mazurak[5]) and Henin[4]), was prevented in the present experiments by the complete evacuation of the samples prior to their removal from the fumigation vessel. The average values for the different soil lots, averaged over the five treatments, are presented in

Table II as a matter of general interest and because they show that the differences between soil lots are not obscured by the effect of the treatment, in spite of the interaction shown in Table I.

Experiment II. Soil Sterilized, Wetted, Air-Dried and Analyzed. The outcome of the experiment II was entirely different from that of Experiment I, as is shown by the analysis of variance of the data, presented in Table III. Due to the one cycle

TABLE III

Analysis of variance of values for mean weight-diameters in mm obtained in experiment II on the influence of sterilization on aggregation of five different soil lots

Source of variance	Degrees of freedom	Sum of squares	Mean square
Methods of sterilization	4	0.0743	0.0186
Soil lots	4	29.7424	7.44 *)
Methods × Soil lots	16	0.7359	0.0460
Error	25	0.7939	0.0318
Total	49	31.3465	—

*) Highly significant (1% probability).

of wetting and drying the differences between the different methods of sterilization, including the one where steam was used, had no statistical significance at all. At the same time the differences between the soil lots were fully maintained. These facts are also apparent from the breakdown into average values for the treatments and the soil lots as given in Table II. From the error component in Table III it may be computed that the least significant difference at the 5% level between two averages is 0.164 mm, all differences being smaller than this value *).

Experiment III. Soil Sterilized, Wetted, Incubated, Dried and Analyzed. The results of the third experiment, presented in Table IV, show a highly significant effect on aggregation due to the different methods of sterilization. The interaction between the treatment and the individual soil lots is

*) Conclusions drawn from the F-test on the mean squares and of t-tests on differences of average values are not of necessity the same. See for a recent discussion: J. W. Tukey, Comparing individual means in the analysis of variance. Biometrics **5**, 99–114 (1949).

TABLE IV

Analysis of variance of values for mean weight-diameters in mm obtained in experiment III on the influence of sterilization on aggregation of five different soil lots

Source of variance	Degrees of freedom	Sum of squares	Mean square
Methods of sterilization	4	2.683	0.716 *)
Soil lots	4	30.054	5.51 *)
Methods × Soil lots	16	2.872	0.179 †)
Error	25	1.611	0.0644
Total	49	37.420	—

*) Highly significant (1% probability).
†) Significant (5% probability).

also seen to be of statistical significance. It seems upon comparing Table III with Table IV that the effects, which were obliterated by a single wetting and drying cycle, have reappeared as a result of the incubation. However, as is shown in Table II, the effect has an entirely different character than was found in experiment I. In experiment III the treatments with 5 and 10 in. of mercury pressure of ethylene oxide gave both highly significant, and almost equal deviations from the control, but no effect was noted for the treatment with 15 in. The effect of heat sterilization appears to be about the same in experiments I and III. It was calculated that the least significant difference at the 1% level between two averages in table II was 0.325 mm.

It is to be noted in column III in the lower part of table 2 that the value for the soil in meadow of corn-oats-meadow is too low as compared with the corresponding values in columns I and II. The low value is thought to be due to erroneous sub-sampling, in which lots with smaller aggregates were preferentially obtained. The results of the experiment itself, that is, the effects of different methods of sterilization are not influenced by this shortcoming. The other values in column III are well in accordance with those presented in columns I and II.

Experiment IV. Soil Sterilized, Wetted, Reinoculated, Incubated, Dried and Analyzed. As is shown in Table V all significant effects attributable to different methods of sterilization have disappeared as the result of reinoculation. This conclusion is fully sustained by the magnitude of the

TABLE V

Analysis of variance of values for mean weight-diameters in mm obtained in experiment IV on the influence of sterilization on aggregation of different soil lots

Source of variance	Degrees of freedom	Sum of squares	Mean square
Methods of sterilization	4	0.241	0.0602
Soil lots	4	27.581	6.89 *)
Methods × Soil lots	16	0.952	0.0595
Error	25	1.053	0.0421
Total	49	29.827	—

*) Highly significant (1% probability).

differences between average values for treatments as given in Table II. All of the differences are smaller than the least significant difference at the 5% level, which is 0.192 mm.

The low value in column four of Table II, for meadow of corn-oats-meadow, must be explained in the same manner as in the preceding section on experiment III, i.e., by faulty subsampling procedure.

Conclusions. The effect of sterilization of soil with ethylene oxide and with heat on the distribution of water-stable aggregates is not as clear-cut, as it was hoped to be at the outset of the described experiments. In cases where sterilization with heat had no perceptible influence on aggregation, there was likewise no influence which could be attributed to ethylene oxide treatment, regardless of the concentration. When soil was wetted and immediately thereafter air-dried or when it was reinoculated after sterilization, the soils remained unaltered by sterilization with heat and by any of the tested rates of ethylene oxide. The findings from the latter case, experiment IV, are of special interest because reinoculating sterilized soil is a common technique in studies on relations between aggregation and microbiological activity. In the described experiments, however, a mixed microbiological population was employed and therefore no assurance exists that the conclusion drawn would hold for pure culture inoculation.

When a soil is sterilized as for subsequent incubation with certain compounds or materials, the experimental results show that both ethylene oxide and heat sterilization have large effects and are

therefore both unsatisfactory. The highest rate of ethylene oxide used constitutes an exception. The differences found between reinoculated soils and sterile soils can probably be attributed only to the activity of microorganisms in the reinoculated soils.

Summary

A study was made of the effect on water-stable aggregate distribution of sterilizing soil at three rates of application of ethylene oxide and with heat in the usual manner. Unsterilized soil was used as a basis of comparison.

Aggregation was increased by heat and the two highest rates of ethylene oxide, when soil was sterilized in the air-dry state and analyzed without any further treatment. When a wetting and drying cycle was added to the treatment no significant effects on aggregation could be found.

Aggregation was also increased by heat and by the two lowest rates of ethylene oxide, when soil was wetted and kept at the moisture equivalent under sterile conditions in an incubator for two weeks. The highest rate of ethylene oxide gave no effect in this case. No differences in aggregation were found when the wet soils were incubated after reinoculation with a mixed population.

It appears that ethylene oxide is by no means an ideal sterilizant for aggregation studies. Fairly large quantities are needed to obtain sterilization. Furthermore, use of ethylene oxide results under certain conditions in significant effects on aggregation. In several instances the effects are comparable to effects attributable to heat.

The results of this experiment also indicate that conclusions drawn from experiments where heat sterilized soils are employed can be erroneous if the effect of the sterilization itself is not accounted for.

Résumé

Une étude fut conduite sur l'effet de la stérilisation du sol sur la distribution des agrégats stables à l'eau. Trois doses différentes d'oxyde d'éthylène furent usées pour la sterilisation de même que la methode ordinaire avec chauffage dans une autoclave. De la terre non stérilisée fut usée comme témoin.

L'agrégation s'accrût à la suite du chauffage et des deux doses les plus élevées d'oxyde d'éthylène, quand le soil sèché à l'air fut sterilisé et analysé sans autre traitement. Quand un cycle de mouillage et de dessiccation fut ajouté au traitement, aucun effet significatif ne fut trouvé.

L'agrégation s'accrût à la suite du chauffage et des deux doses les plus basses d'oxyde d'éthylène, quand le sol fut mouillé jusqu'au "moisture equivalent" et fut incubé aseptiquement pendant deux semaines. La dose la plus élevée d'oxyde d'éthylène n'eut aucun effet dans ce cas. Quand le sol fut ensemencé avec une population mixte avant l'incubation, aucun effet sur l'agrégation ne fut constaté.

Il apparait que l'oxyde d'éthylène n'est pas un stérilisant idéal pour des

études d'agrégation. Des quantitées assez grandes sont necessaires pour obtenir la stérilité. De plus, l'usage d'oxyde d'ethylène a, dans certaines conditions, des effets sur l'agrégation. Quelquefois ces effets sont comparables aux effets dûs à la stérilisation par chauffage.

Les résultats des expériences rapportées indiquent aussi que des conclusions tirées des experiences sur des sols sterilisés par chauffage, peuvent être erronées, si l'effet propre de la stérilisation comme telle n'est pas pris en considération.

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LITERATURE CITED

1) Allison, L. E., Effect of microorganisms on permeability of soil under prolonged submergence. Soil Sci. **63**, 439–450 (1947).
2) Clark, F. E., The use of ethylene oxide for soil sterilization. Fourth Int. Congr. Soil Sci. (in press).
3) Dalton, F. H. and Hurwitz, C., Effect of volatile disinfectants on survival of microflora in soil. Soil Sci. **66**, 233–238 (1948).
4) Henin, S., Essay d'une théorie de la stabilité de la structure des sols. Plant and Soil **1**, 167–178 (1948).
5) Mazurak, A. P., Effect of gaseous phase on water-stable synthetic aggregates. Soil Sci. **69**, 135–148 (1950).
6) Roberts, J. L., Allison, L. E., Pickett, P. S., and Riddle, K. B., Preliminary studies on soil sterilization with ethylene oxide. J. Bact. **45**, 40 (1943).
7) Van Bavel, C. H. M., The mean weight-diameter of soil aggregates as a statistical index of aggregation. Soil Sci. Soc. Am. Proc. **14**, (1949) (in press).
8) Yoder, R., A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. J. Am. Soc. Agron. **28**, 337–351 (1936).

Plant and Soil II, no 4 December 1950

CONDUCTIVITY AND BUFFER CAPACITY OF POTATO JUICE AS CORRELATED WITH POTASSIUM SUPPLY TO THE PLANT

by A. H. A. DE WILLIGEN

Exp. Sta. for the Industrial Use of the Potato, Groningen

1. *Introduction.* In a previous article [1]) we described how a survey of electrical conductivity of potato juice formed the basis of a method for the determination of potassium content of the potato. In connection with research on the manufacture of potato protein we also determined the quantity of sulphuric acid necessary to bring the pH of the juice to 4.3, the optimal value for the coagulation of tuberin.

For practical purposes we made both measurements on the same potato samples and noted a correlation, which could be predicted from theoretical considerations. The conductivity of plant juice is, in the first place, determined by the hydrogen ion concentration, which however in the potato is practically constant in widely diverging circumstances. Hence any variation is therefore principally due to a second factor, the concentration of potassium ions. All other ions have a minor influence only in this respect, because of their low concentration or small conductivity number. Variations in the buffer capacity might be expected to correlate with variations in the phosphoric acid content and content of organic acids. A relationship between these values and that of the potassium ion has been previously noted [2]).

From the work of van Itallie [3]) we know, that the potassium ion concentration in the potato is a function of the potassium supply to the plant, i.e. of soil conditions and of the supply of fertilizers. Although the following data do not present any new principle, our choice of simple and rapid electrical methods has given us the opportunity of amassing much more data than van Itallie, so that we have been able to study more details.

We are indebted to the Agr. Exp. Sta. and Institute for Soil Research T.N.O. at Groningen for the potato samples grown during their survey of the magnesium condition of peaty soils in 1947 (registration number Pr 970). The fields on the latter soils are in many respect a good sample of the whole area of older potato soils in the S.E. of the province of Groningen. Other samples were taken from the potassium fertilizer experiments of the same Station and from experiments carried out by Ir K. B. Kluiving at Emmen and by Ir D. J. Pattje at Veendam.

2. *Experimental methods.*

About 5 to 10 kg potatoes were pulped in a small scale factory mill. The pulp was centrifuged through cotton cloth into a 500 cc Erlenmeyer flask, in which the last grains of starch settled. About 25 cc were placed in a 50 cc beaker in a thermostat bath at 25° ± 0.2°. As soon as the juice had reached the right temperature the electrical conductivity was determined with the Philoscope. It proved to be very important to finish the determination within half an hour after pulping, for later the conductivity increased very rapidly. Indeed after 24 hours we often found an increase of 50%. This could have been caused in two ways: The juice is very heavily infected by lactic acid bacteria and its pH often changes from 6 to 3.5 within 24 hours. Again amides are gradually changed to ammonium salts. Both changes affect the conductivity in the same sense.

The buffer capacity was measured by titration of 20 cc of starch-free juice diluted to 200 cc with dist. water, using the Cambridge electrotitrator with glass electrode. The original pH of the diluted juice was 5.8–6.2. We titrated with 0.1 N sulphuric acid to an end point at 4.3. At this point the accuracy of the potentiometer was 0.02 pH, corresponding with $1–1^1/_2$ milli-equivalent/l of acid.

The major source of error in this estimate was caused by flocculation of protein on the glass electrode. High room temperatures in Sept. 1947 with a corresponding activity of micro-organisms increased this error. On finishing a series of measurements the glass electrode was calibrated with a buffer solution. Several series of measurements had to be discarded because incorrect values were found by this calibration.

3. *Conductivity of juice, potassium content of tubers and potassium number of the soil.* The simplest example of a correlation between potassium supply and conductivity was found on a uniform experimental field with different potassium applications.

On field no Pr 201 at Pieterzijl for about 15 consecutive years applications of 7 different amounts of chlorine-free potassium salts have been given on 21 plots, all other fertilizer conditions being

kept optimal. The different doses of potassium fertilizers caused the same clearly visible reactions in 1947 as in previous years. On plots without fertilizer the well known bronze leaf colours appeared. The highest amounts of potassium caused stiff leaves and gave other symptoms of excess (including low specific gravity of the tubers). The differences in production caused by any heterogeneity in the field were low compared with those caused by the potassium treatments. Differences between samples from this field might therefore be expected mainly due to the different potassium applications. A high correlation between amount of fertilizer and potassium content of the tuber was found.

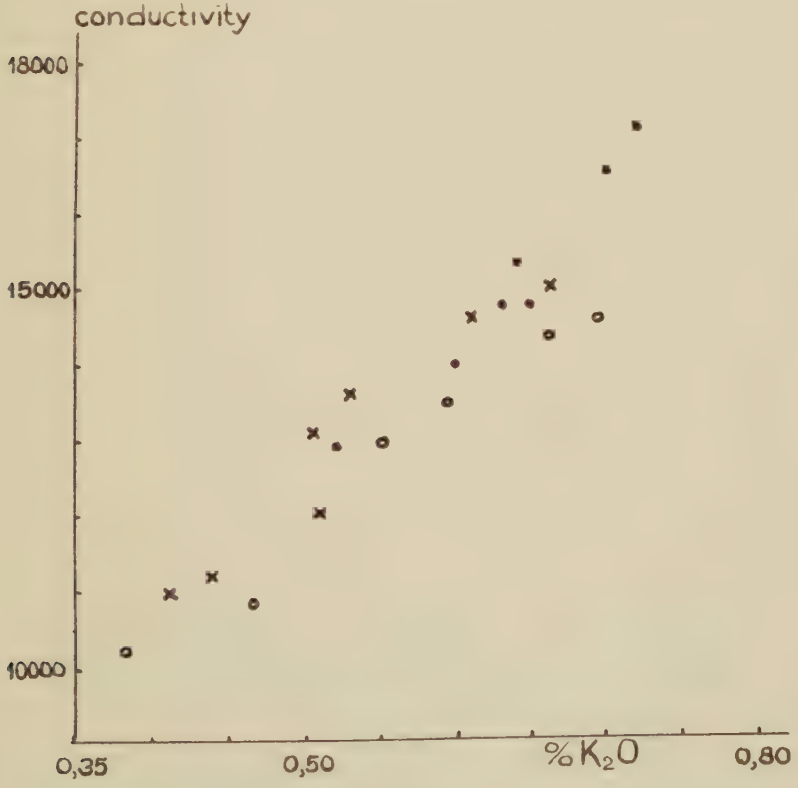


Fig. 1. Conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}) and potassium conrent of the tuber in samples from exp. field Pr 201 at Pieterzijl, harvest 1947

.... variety Rode star

× × = Eigenheimer

o o = Noordeling

In fig. 1 conductivity is plotted against potassium content. The correlation is high; the points form a straight line. The minor spreading of the points around the line partly originates from the fact that three potato varieties were used on different plots. The points of the variety Rode Star lie above the line, those of Noordeling beneath it. This experiment cautioned us against the simultaneous use of different varieties and probably a separate curve should be made for each variety. Fig. 2 gives the same values for another experiment (Pr 100 at Emmercompascuum). This figure matches fig. 1 for the points of Pr 100 lie between those of Pr 201.

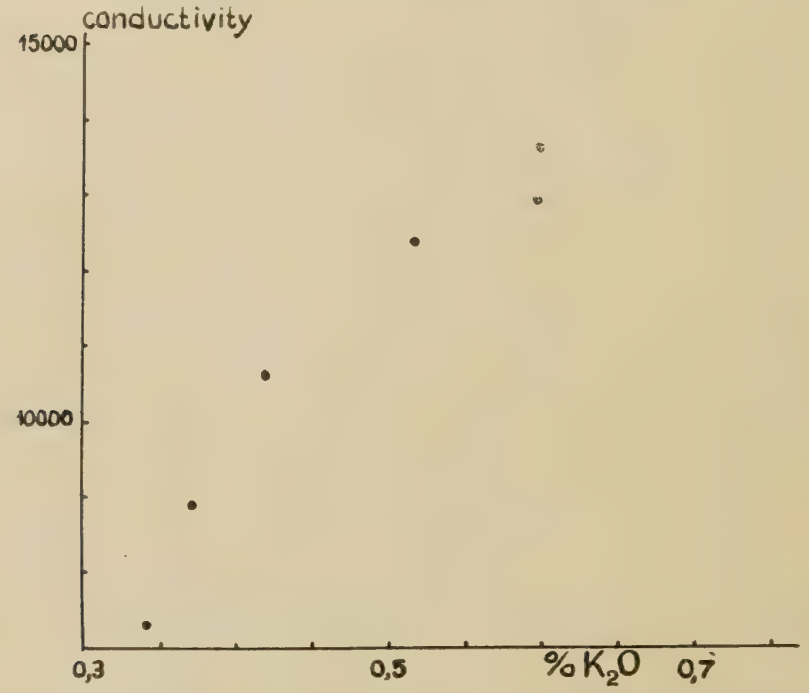


Fig. 2. Conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}) and potassium content of the tuber in samples from exp. field Pr 100 at Emmercompascuum, harvest 1947.

In fig. 3 the conductivity is shown as a function of the potassium number of the soil. This correlation is also very high. Conductivity is less influenced by changes at the higher potassium numbers than at the lower ones (which is the common case in all considerations of the composition of plants).

The above figures came from carefully picked experiments, in which all other variables were kept as constant as possible. The following data relate to normal fields. These were chosen from one region (peaty, sandy soils in the old moor colonies of the province of Groningen), on which was cultivated one variety only (Ultimus). In other respects we chose very divergent conditions, e.g. in humus content and pH of the soil, in planting time and in seed potato

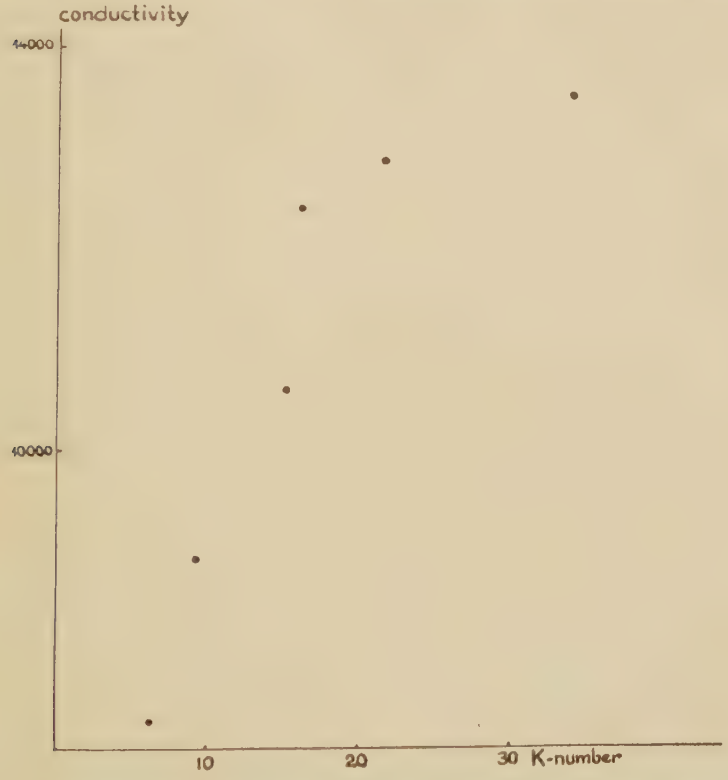


Fig. 3. Conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}) and K-number of the soil of exp. field Pr 100 at Emmercompascuum, harvest 1947.

quality and dimensions. The usual potassium dressings were given, in the form of chlorinated potassium salts, which, because of late delivery, were applied in many cases in spring, even as late as May.

Fig. 4 gives the parallel of fig. 1 and 2 for 58 samples of this group. In spite of the variation in conditions of growth the correlation is still remarkably good. In one respect, however, there is a difference

from fig. 1. The line through the points cannot be a straight line, but is curved. How does this curvature come about?

In the first place some of the juices had a considerable chloride content caused by the form of potassium dressing. With some uncertainty we drew a line through figure 4, as an auxiliary for statistical treatment. The distances of the separate points from this line were brought into an auxiliary figure (not published here) as a function of the chloride content of the tuber. This function was a straight line. By use of this auxiliary figure the auxiliary line in fig. 4 was corrected, and a better figure for the influence of chloride was

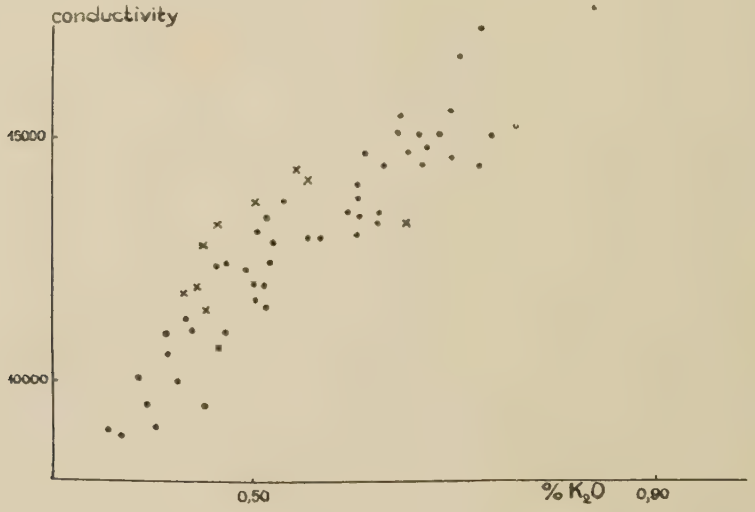


Fig. 4. Conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}) and potassium con tent of the tuber for samples from 60 fields, harvest 1947.

obtained (fig. 5). The conductivity is a straight line function of the chloride content of the sample (and thus of the juice). The inclination of the line compares favorably with the theoretical value calculated from the known transportnumber of the chloride ion (which is very high compared to that of the organic ions, that are displaced by chloride).

A comparable statistical treatment was given in respect to the phosphoric acid ion. After correction for potassium and chloride ions together we obtained fig. 6, which is quite remarkable. We must conclude from this figure that in the case of lack of phosphorus the

plant can fill the gap with other ions of greater conductivity. Perhaps organic acid ions of low molecular weight, or sulphate or, in extreme cases, also nitrate (in normal mature tubers this last substance is practically absent) could play this rôle.

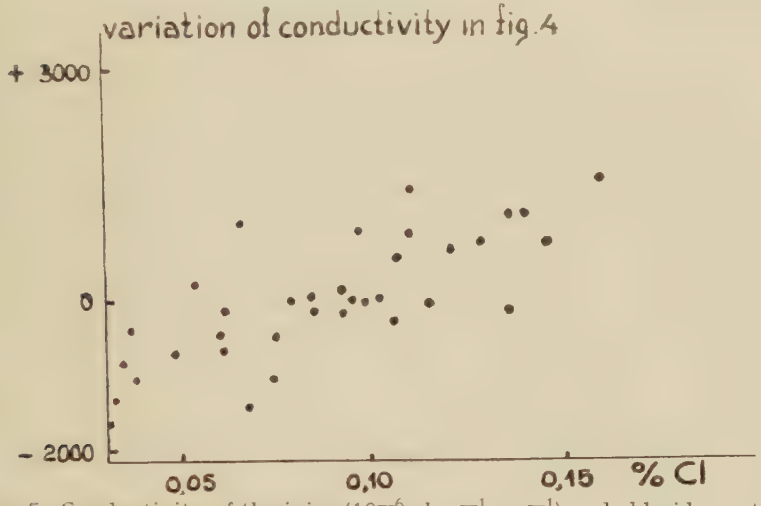


Fig. 5. Conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}) and chloride content of the tuber. The conductivity values are corrected for the influence of the potassium content of the tuber.

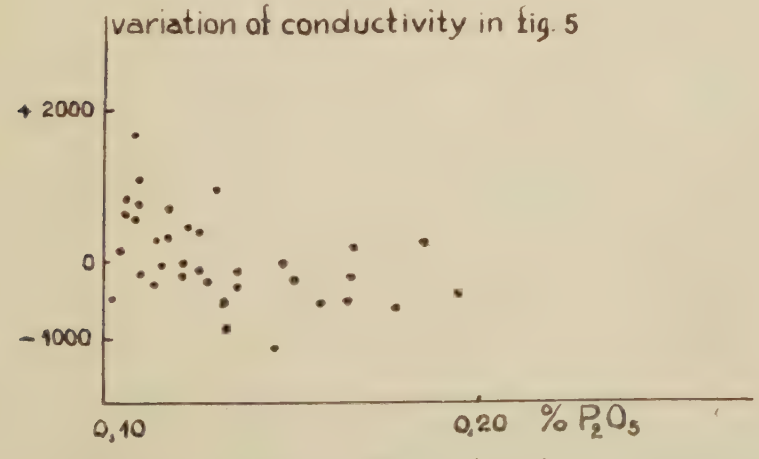


Fig. 6. Conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}) and phosphorus content of the tuber. The conductivity values are corrected for the influence of the potassium content and of the chloride content of the tuber.

With the corrections found in this way we return to graph no 4 and eliminate all samples, in which too high a chloride content was found (crosses). The other points have a much higher correlation. Thus the conductivity of the juice is a good measure for the potassium content, except when too much chloride has been given or when a lack of phosphate has occurred. Nevertheless all the corrections together fail to straighten the line. The left part of the figure is still curved, and for this we have found no reason in the present study.

A group of 82 samples was used to correlate conductivity and potassium number of the soil. The potassium number is obtained by extraction of variable amounts of soil containing 6.25 g humus with the same amount of hydrochloric acid. It is know that in peaty soils there is an influence of this varying proportion in the result, so that potassium numbers should be compared in soil samples of approximately equal humus content only. This is done in fig. 7.

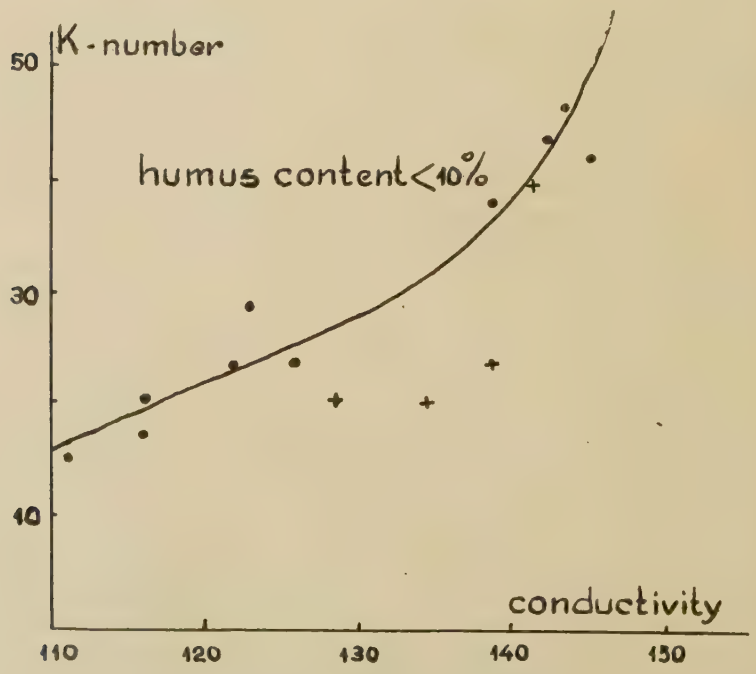
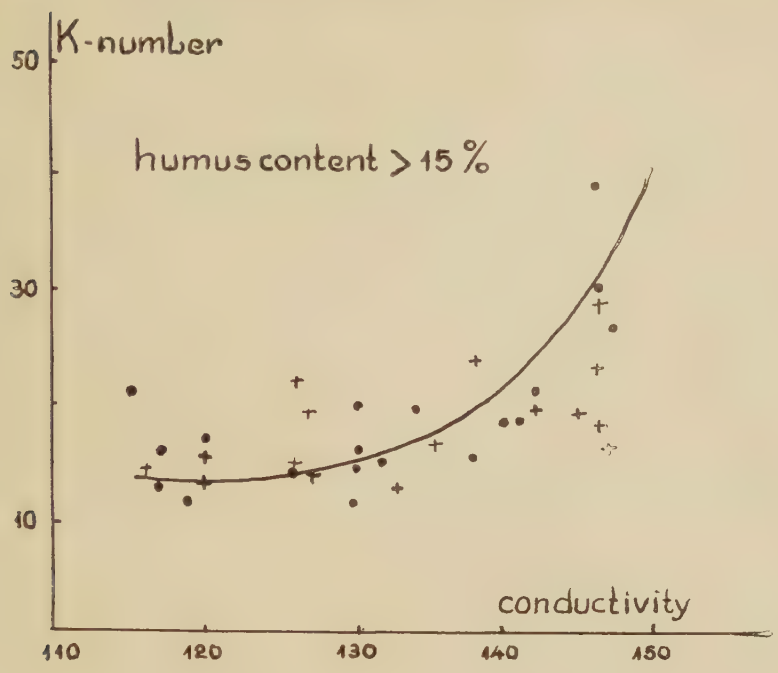


Fig. 7. K-number of soils and conductivity of the juice of the tubers. The soils are classified in three groups according to humus content.


K-number
50
30
10
humus content > 15 %
conductivity
110
120
130
140
150

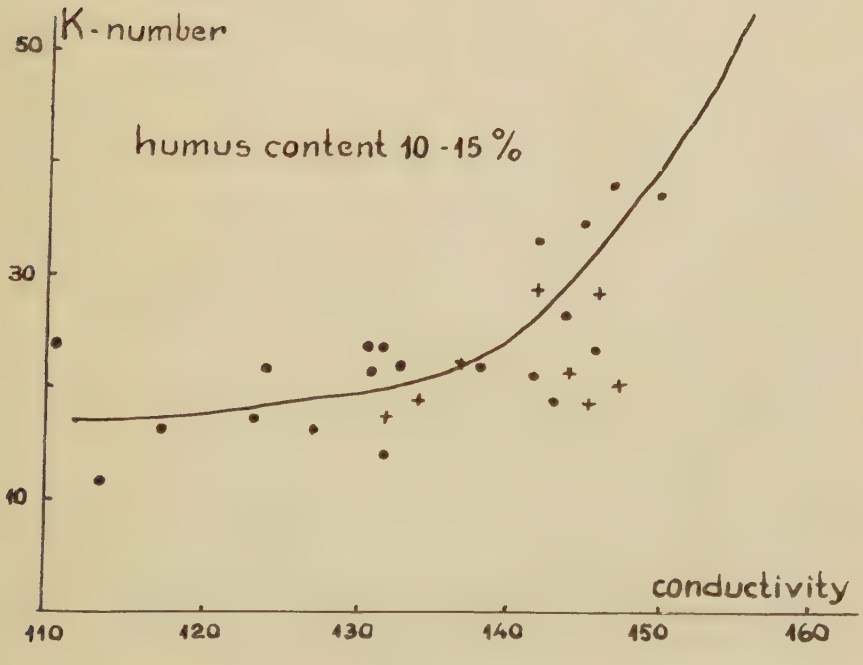

K-number
50
30
10
humus content 10 -15 %
conductivity
110
120
130
140
150
160

Fig. 7

In all groups of approximately equal humus content a certain proportionality between conductivity and *K*-number is found. With lower *K*-number however very different conductivities can be found. A more detailed study would have to account for other variables also, e.g. fertilizer application and pH of soil. This would lead outside the scope of our investigation and of the working programm of our Institute. A publication of the Agr. Exp. Sta. and Institute for Soil research T.N.O. is in preparation.

We notice however, that in some cases the yield of dry matter corresponded somewhat better with the conductivity than with the *K*-number. Therefore the suggestion is made that conductivity determination should be tried on a much more elaborate scale for the estimation of soil conditions. This could be done very easily in this part of the Netherlands because practically the whole potato harvest is pulped for starch manufacture.

In one respect however fertilizer application has a big influence. Chloride of potassium applied in April and May brings about a higher uptake of both potassium and chlorine (van Itallie[3])) and gives a much higher conductivity. Even in this case, the increased conductivity does give a certain insight into the conditions of the plant, of the soil and its manural status.

4. *Conductivity and buffer capacity of the juice.* There is also a close relationship between conductivity and buffer capacity. In table I we give an example from one experimental field (Zgr 148, Borgercompagnie, harvest 1948). With increased potassium applications as sulphate both values go up. The higher buffer capacity is not caused by an increased phosphate uptake. On the contrary this uptake decreases from 0.16% to 0.13% or 0.14%. This lower intake is more than compensated by intake of chloride and sulphate, but at the same time the potassium concentration has increased much more. Hence an extra amount of organic acids must have been formed.

In the field a series of KCl-dressings were given. We chose two samples of the ripe harvest of such plots, and of these analyses are given in tab. I. In accordance with the concept of the ionic concentrations in the tuber given by van Itallie we expected that conductivity would increase more than with the corresponding potassium sulphate dressings, but that the buffer capacity would either

not increase, or would increase much less rapidly. Table I confirms this.

TABLE I

Buffer capacity and conductivity of the juice of samples from the potassium experiment Zgr 148 at Borgercompagnie, harvest 1947

Sample	Object	Buffer capacity milli-equivalent	Conductivity $\times 10^{-6}$ Ohm^{-1} cm^{-1}	Analysis of whole tuber			
				P_2O_5 %	Cl %	SO_4 %	sum in milli-equivalent/100 g
6075	K_2SO_4	52	8 200	0.16	0.12	0.47	15.7
6072	165 kg K_2O	81	13 600	0.13	0.20	0.54	18.6
6071	330 kg K_2O KCl in spring	90	15 200	0.14	0.21	0.66	20.4
6075	0 kg K_2O	52	8 200	0.16	0.12	0.47	15.7
6073	165 kg K_2O	70	14 200	0.14	0.43	0.50	24.8
6074	330 kg K_2O	69	15 400	0.13	0.63	0.46	29.2

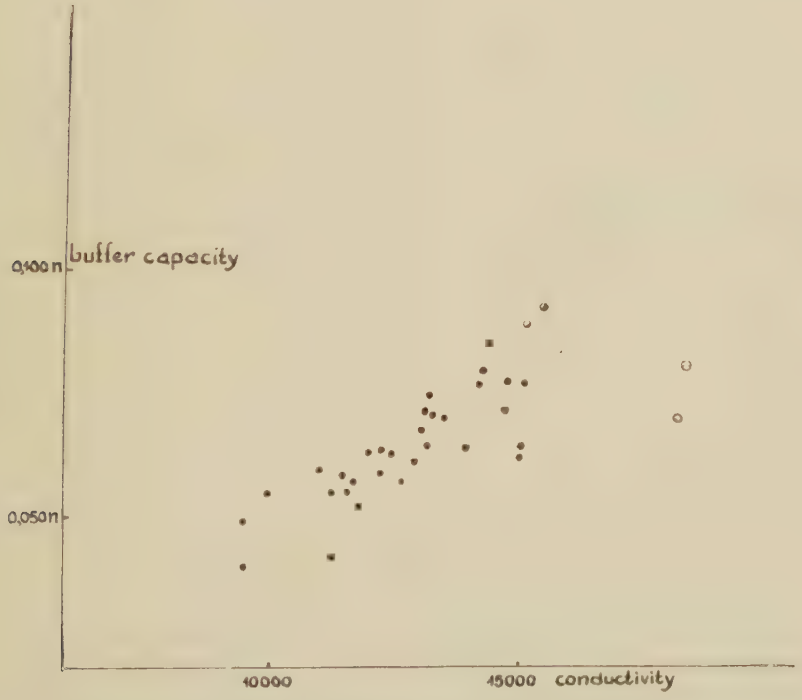


Fig. 8. Buffer capacity and conductivity of the juice (10^{-6} ohm^{-1} cm^{-1}). The values for the buffer capacity are corrected for the influence of the chloride content of the tuber.

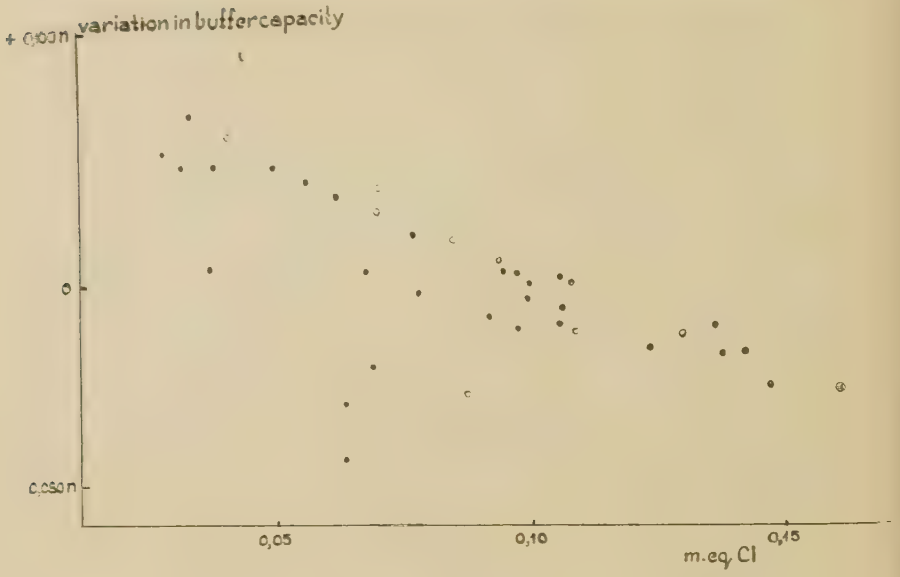


Fig. 9. Variation in the buffer capacity caused by the varied chloride content of the tuber. The buffer capacity values are corrected for the influence of the conductivity (i.e. in an indirect way for the potassium uptake of the plant).

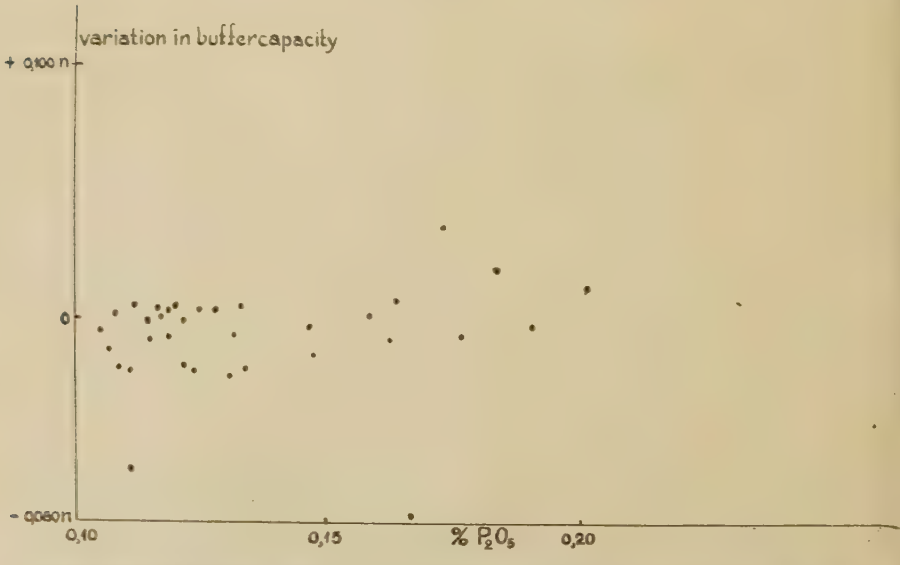


Fig. 10. Variation in the buffer capacity as a function of the phosphorus content of the tuber. The buffer capacity values are corrected for the influence of the conductivity and of the chloride content of the tuber.

We obtained similar results with samples of EC 43, Emmercompascuum harvest 1947.

Finally, in Figs. 8, 9 and 10 are given a survey of a number of fields from the same area as in fig. 3. By means of a statistical treatment in graphform like that of the conductivity measurements we tried to eliminate the influence of chloride and phosphate content. This succeeded very well. The decline of the buffercapacity in fig. 9 is almost equivalent to the rise in chloride content. The phosphate content does not have any real significance in this respect. With lack of phosphate the formation of an equivalent amount of organic acid is evident.

After correction for the influence of chloride, fig. 8 gives a strong positive correlation between buffer capacity and conductivity. The buffer capacity is therefore determined by the potassium uptake of the plant except when KCl has been given very late.

This result again demonstrates the great influence of the potassium supply on the physiology of the potato. The exceptional place of potassium chloride is also indicated anew.

Summary

Electrolytic conductivity and buffer capacity of the press juice of the potato tuber are almost exclusively determined by the potassium supply to the plant and by the form in which the potassium fertilizer is given (especially in respect of the chlorine uptake of the plant).

Received April 10, 1950.

REFERENCES

1) van Itallie, Th. B., Over de chemische samenstelling van aardappelen in verband met de kalibemesting. Landbouwk. Tijdschr. **45**, 241–255 and 421–436 (1933) and **46**, 272–286 (1934).
2) de Willigen, A. H. A., and Reestman, A. J., Rassenproeven met fabriksaardappelen. Verslag Landbouwk. Onderzoek **50**, 619–653 (1950).
3) de Willigen, A. H. A. and de Groot, P. W., Schattingsmethode voor het kaligehalte van aardappelen door meting van het geleidingsvermogen. Landbouwk. Tijdschr. **59**, 217 (1947).

LETTER TO THE EDITORS

The effect of Manganese on the Catalase activity of Rice Plant

Inspite of the necessity of manganese for the growth and development of plants, the physiological role of the said element is little understood. The present investigation was planned to study the effect of the said micronutrient on the catalase activity of rice plant.

Rice 'Seeds' CH45, supplied by the Central Rice Research Institute, Cuttack were sterilized by soaking for 10 minutes in a 1 : 1000 solution of bichloride of mercury. The seeds were washed several times in water. They were then germinated in sand and the seedlings (height $7 \pm .5$ cm) were transferred to the culture solution.

The culture vessels were two litre 'pyrex' beakers, covered with thick black papers to keep the roots in dark. Each vessel was fitted with a thickly paraffined cover having ten holes to accommodate plants. Two plants were placed in each hole and were held in position with tufts of cotton. The composition of the basic nutrient solution was as follows: KH_2PO_4 — 1.25 g, $Ca(NO_3)_2$ $4H_2O$ — 4.27 g, $MgSO_4$ $7H_2O$ — 2.28 g, $(NH_4)_2SO_4$ — 0.38 g and 1 ml of 1% Fe tartarate Sol in one litre of distilled water. Besides using 'extra pure' chemicals, no special method of purification of the culture solution was adopted. H_3BO_3 and $CuCl_2$ were added to the culture solution in concentrations of .5 ppm B and .01 ppm Cu respectively. The treatments were no added manganese and manganese (as $MnCl_2$) added in concentrations of 1, 5, 10, 20 and 50 ppm Mn.

The determination of the catalase activity was made when the plants were 10 weeks old and morphological differences due to different doses of manganese were clearly evident. Plants were removed between 11.30 A.M. and 12 Noon and the enzyme activity was determined immediately after it. For the said purpose, mature leaves were selected and in case of root, the growing region was used for analysis.

For the determination of the said enzyme, gasometric method was used and the general procedure was the same as used by the author [2]), [3]). The results, obtained have been presented in Figure 1 as cc of oxygen collected from standard hydrogen peroxide solution at the end of 5 minutes by macerated extract corresponding to 0.1 g of plant material.

Baily and Mc. Hargue[1]) report that even 1 ppm of added manganese depresses the catalase activity in alfalfa. The present findings on the contrary show that addition upto 10 ppm of manganese increases the said enzyme activity. The depression in the catalase activity in case of alfalfa after the addition of 1 ppm of manganese may be due to the very low requirement of manganese by the said plant in comparison to rice. Earthenware pots containing acid washed sand have been used for growing of alfalfa plant by Baily and Mc. Hargue. It is likely, that manganese present in the medium as impurities was high and after the addition of 1 ppm Mn, it was toxic to the catalase activity. The possibility of manganese in suitable concentrations increasing the catalase activity has

not been ruled out by the said authors (1). It is therefore, probable that manganese through the direct influence on the iron containing prosthetic group controls the enzyme (catalase) activity.

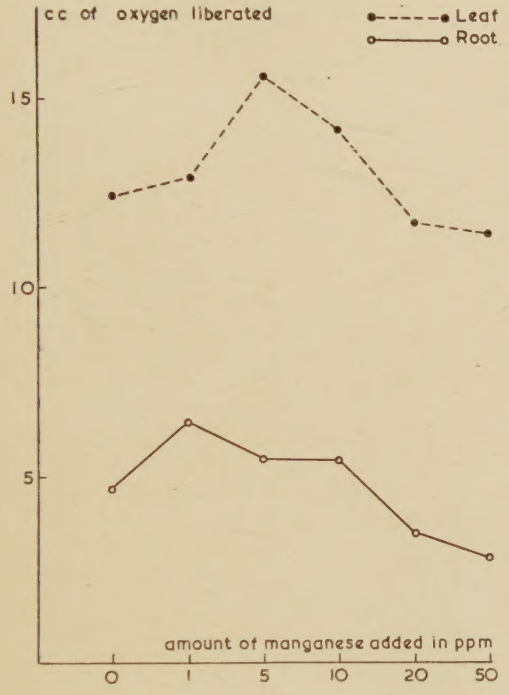


Fig. 1

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REFERENCES

1) Bailey, L. F. and Mc. Hargue, J. S., Effect of boron, copper, mangenese and zinc on the enzyme activity of tomato and alfalfa plants grown in the green house. Plant Physiol. **19**, 105 (1944).
2) Pattanaik, S., A comparative study of the catalase activity of the petals and leaves of *Hibiscus rosa sinensis*. Current Sci. **18**, 212 (1949).
3) Pattanaik, S., The effect of boron on the catalase activity of rice plant. Current Sci **19**, 153—154 (1950).

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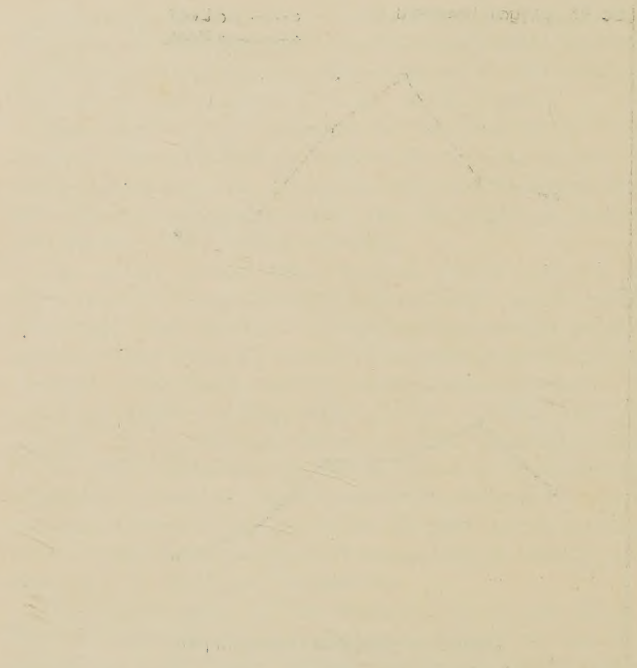

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